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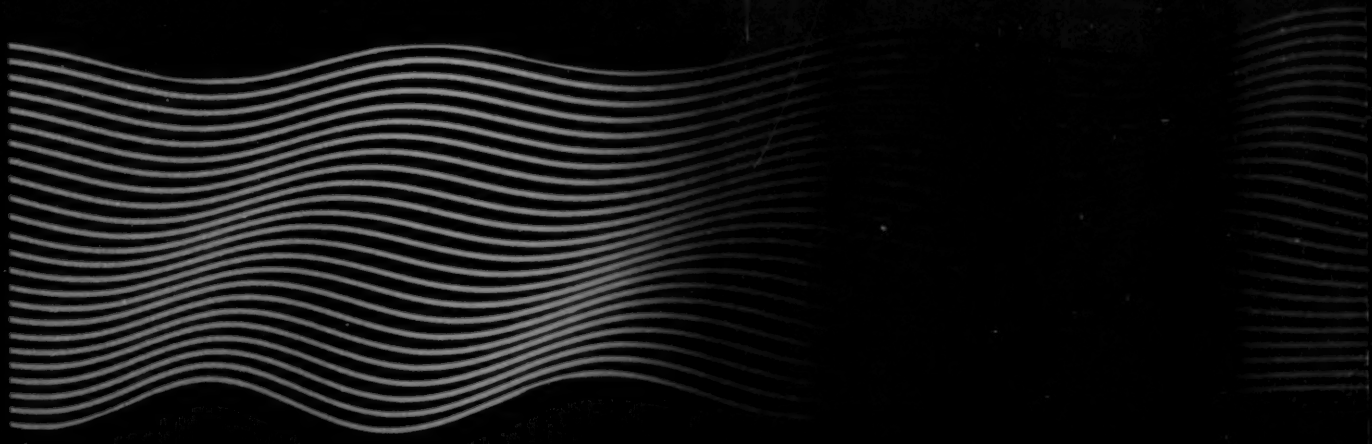

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Ocean survival and exploitation of coho salmon (*Oncorhynchus kisutch*) stocks from the east coast of Vancouver Island, British Columbia

Marc Labelle, Carl J. Walters, and Brian Riddell

Abstract: Juvenile tagging and escapement enumeration was conducted during 1985–1988 in nine streams within a 150-km section on the east coast of Vancouver Island. Fourteen coho salmon (*Oncorhynchus kisutch*) stocks of wild, hatchery, and mixed origin were monitored for ocean survival and exploitation patterns. Estimates of smolt-to-adult survival ranged from 0.5 to 23.1%. Survival rates were highly variable across years and stocks. No stock or stock type had consistently higher survival, but one hatchery stock exhibited consistently lower survival. Average exploitation rates were about 80% each year, and were as high as 96% for some stocks. Exploitation rates were not consistently higher or lower for any stock or stock type, but hatchery reared coho tended to be subject to higher exploitation. Log–linear models were used to assess the effects of various factors on survival and exploitation. Certain hatchery rearing practices had a large influence on survival. Genetic factors, run timing, and stream location had large influences on exploitation rates. An assessment of covariation in survival and exploitation rates showed no indication of a high level of similarity among stocks from adjacent streams or among stock types in this region.

Résumé : De 1985 à 1988, des opérations d'étiquetage de juvéniles et de dénombrement d'échappées ont été effectuées dans neuf cours d'eau répartis sur 150 km du littoral est de l'île Vancouver. Quatorze stocks de coho d'origine sauvage, d'élevage et mixte ont été suivis pour déterminer leur taux de survie dans l'océan et d'exploitation. Les estimations de taux de survie varient de 0.5% à 23.1%. Les taux de survie varient fortement entre année et entre stocks. Aucun stock ou type de stock ne montre de taux de survie constamment supérieur aux autres, mais un stock d'élevage a un taux de survie toujours inférieur aux autres. Les taux d'exploitation moyens étaient d'environ 80% chaque année, et ont atteint 96% pour certains stocks. Les taux d'exploitation n'étaient pas constamment maximum ou minimum pour aucun stock en particulier, mais les coho d'élevage avaient tendance à être sujets à des taux d'exploitation plus élevés. Des modèles log-linéaires ont été utilisés pour évaluer les effets de différents facteurs sur la survie et l'exploitation. Certaines pratiques d'élevage ont une influence prononcée sur les taux de survie. Le patrimoine génétique,le moment de la remonte, et la localisation du cours d'eau ont une influence prononcée sur les taux d'exploitation. Une évaluation de la covariation des taux de survie et d'exploitation n'a pas indiqué une forte similarité entre les stocks de cours d'eau adjacents ou d'un certain type dans cette région.

Introduction

Fishing mortality exerts selective pressures on populations. This selection is influenced by the distribution of the fish in relation to the fishermen, the behavioral response of the fish to the gear, and the mechanical selection of the gear for morphological characteristics (Parrish 1963). Such factors, respectively referred to as availability, vulnerability, and gear selectivity, influence the probability of capture among members of each population. This implies that populations exhibiting different behavioral and morphological attributes may be subject to different exploitation patterns and ocean survival rates.

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Numerous investigations have documented the biological adaptations of Pacific salmonids to local conditions (Helle 1981). Adaptations such as adult body size, run timing, and marine distribution are known to influence subsequent exploitation (McDonald 1981). Less obvious factors, such as smolt size and migration, can also influence subsequent exploitation and contributions to selected fisheries (Novotny 1980; Bilton et al. 1982; Fedorenko and Perry 1984).

With the introduction of hatcheries, a new assortment of rearing environments has emerged. This lead to the proliferation of populations with peculiar life history traits that can influence their subsequent survival and susceptibility to exploitation. Consequently, domesticated and wild strains of freshwater salmonids have been observed to exhibit differences in survival (Schuck 1948; Miller 1951; Mason et al. 1967; Reisenbichler and McIntyre 1977; Fraser 1981; Keller and Plosila 1981), growth rate (Vincent 1960; Reisenbichler and McIntyre 1977), feeding behavior (Rowe 1984), distribution (Vincent 1960), catchability (Flick and Webster 1963), and availability to anglers (Cordone and Nicola 1970). Phenotypic differences were also detected between hatchery and wild strains of Atlantic salmon (*Salmo salar*) (Sosiak et al. 1979; Dickson and MacCrimmon 1982) and Pacific salmon (Glova 1978; Myers 1980; Hjort and Schreck 1982).

Differences between hatchery and wild strains are induced

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by genetic factors and by hatchery experience, but the contribution of each factor is usually not known with certainty. There is evidence that specific hatchery practices alone can influence the exploitation of domesticated stocks (Johnson 1970; Lander and Henry 1973; Hager and Noble 1976; Bilton 1978; Novotny 1980; Bilton et al. 1982, 1984). In some cases, hatchery rearing had little effect on the behavior of the fish after release (MacCrimmon and Hawkins 1976; Webster and Flick 1981), but in others, the effects are so pronounced that the fish behave as different species (Dickson and MacCrimmon 1982).

Despite mounting evidence that hatchery practices can influence the behavior, survival, and subsequent exploitation of salmonids, no studies have shown that certain practices induce significant and consistent differences in exploitation patterns and ocean survival within local assemblages of wild and hatchery salmon stocks. As a result, reference is often made to an "indicator or index stock" when drawing inferences about the demographic traits of salmon populations from a certain region. The following example typifies the approach:

> "We selected four indicator stocks for which adequate coded-wire tag recovery information is available, and which we judged to be representative of the stocks of concern in terms of geographic distribution, age, maturity characteristics and exploitation patterns. All indicator stocks coded wire recovery data were from hatchery releases. These data are considered the best scientific information for describing both wild and hatchery exploitation and contribution. (Anonymous 1983)."

To assess the validity of the assumptions implied in the above statement, a large-scale empirical investigation was conducted during 1984–1988 in southern British Columbia. The primary objectives of this investigation were to test two hypotheses: (*i*) there exists no significant differences among geographically close stocks of coho salmon (*Oncorhynchus kisutch*) in terms of ocean survival, and (*ii*) there exists no significant differences among these stocks in their susceptibility to exploitation. Two conditions should be met to prevent rejection of the latter hypothesis: stocks must contribute to the same fisheries, and if so, they must be subject to the same overall exploitation rate. Since all stocks were subject to coded-wire tagging and escapement enumeration, information was available on stream location, flow patterns, smolt size, time of ocean entry, run timing, adult size, age at return, year of return, exposure to enhancement activities, hatchery rearing practices, genetic background, and ocean distribution. A log-linear model was used to assess the effects of such factors on exploitation and survival rates.

Materials and methods

Nine streams on the east side of Vancouver Island were selected for this study based on accessibility, geographical location, coho abundance levels, hydrological regimes, availability of logistic facilities, and exposure to enhancement activities (Fig. 1). These consisted of the Quinsam River, Black Creek, Puntledge River, Trent River, Rosewall Creek, Big Qualicum River, Little Qualicum River, French Creek, and Millstone River. Details on the biological and physical features of these systems were reported by Hancock and Marshall (1985), Reinhardt and MacKinnon (1979), Fraser et al. (1983), Hamilton (1978), Hurst and Blackman (1988), Lukyn et al. (1985), Bocking et al. (1988), and Labelle (1990*a*).

The different "types" of stocks were classified as follows; "wild" stocks reared under natural conditions, "production" stocks reared under hatchery conditions, "colonization" stocks reared initially hatchery conditions and were released as fry (<10 g) in streams to complete their growth, and "enhanced" stocks comprising a mixture of wild and unmarked hatchery coho from colonization releases. Wild brood stock was also collected in three streams (Black Creek, Trent River, and Little Qualicum River) during two successive years, and the progeny was reared and released from the Rosewall Creek hatchery. This allowed comparisons to be made between hatchery stocks and their natural counterparts of similar genetic makeup. In total, 14 stocks were available for comparative purposes (Fig. 1). Coho from different streams were considered to be genetically distinct, except when coho in one stream consisted of the progeny of spawners taken from another stream. In such cases, the progeny was considered to be genetically similar to the sibling population in the parent stream.

Juvenile tagging and escapement enumeration were conducted in all streams to provide stock-specific information on the traits of interest. Coded-wire tags (Jefferts et al. 1963; Jewell and Hager 1972) were applied to fry and smolts from the 1983 to 1985 brood years. Smolts were tagged during the spring and summer of 1985, 1986, and 1987. Trapping operations were also conducted concurrently in all systems to determine smolt production, emigration patterns, and the number of tagged fry that survived from hatchery outplanting activities conducted previously. Escapement enumeration and tag recovery operations were conducted at the release sites and in adjacent streams during 1985–1988. Labelle (1990*a*, 1992, 1994) provided details on the tagging, escapement estimation, and tag recovery operations conducted in each stream. Additional information on the biological traits of juveniles and adults from each stock were reported by Labelle (1990*b*). The present analysis and results are based on data provided by the release of about 1.5 million tagged smolts, the recovery of about 100 000 tagged adults and jacks, and the use of over 140 distinct tag codes.

Extraction and compilation of tag recoveries by time and area strata

The numbers and identities of tagged coho caught in various fisheries are obtained through an extensive coast-wide sampling program of commercial and sport salmon fisheries in the United States and Canada (Kuhn et al. 1988). The Canadian Department of Fisheries and Ocean (DFO) maintains a data base containing records on tagging procedures, juvenile attributes, the number of tagged fish detected in various samples, and other pertinent factors. All statistics used in the present analysis were extracted from this data base, using the standard procedures described by Kuhn (1988). The stratification level used for data extraction was selected mainly to distinguish the major fisheries characterized by specific season lengths, gear types, sampling regimes and geographic locations.

The number of tag recoveries in catch and escapement strata was estimated using a complex procedure based on the one described by Kuhn (1988). The procedure accounted for commercial sampling rates; voluntary return rates in the sport fishery (the "awareness factors;" Kimura 1976); tag identities; and the number of missing, lost, or unidentifiable tags (or "nontags;" Kuhn et al. 1988). Corrections were also made for apparent errors in awareness factor estimates, escapement sampling deficiencies, and the number of coho that strayed to locations other than their stream of origin, whenever these could be detected. The reader can refer to Labelle (1990*b*, 1992) for a detailed description of this procedure. In the following sections, the estimated number of tags of a certain group, recovered in a given stratum, will be termed "total recoveries," i.e., the observed number of recoveries in a stratum, corrected for identification and sampling problems, and expanded by the reciprocal of the sampling rate. Unless specified otherwise, a group is represented by all tag codes associated with a particular stock and brood year combination. The term "adults" will refer to coho that mature after 1 or 2 years of marine residency

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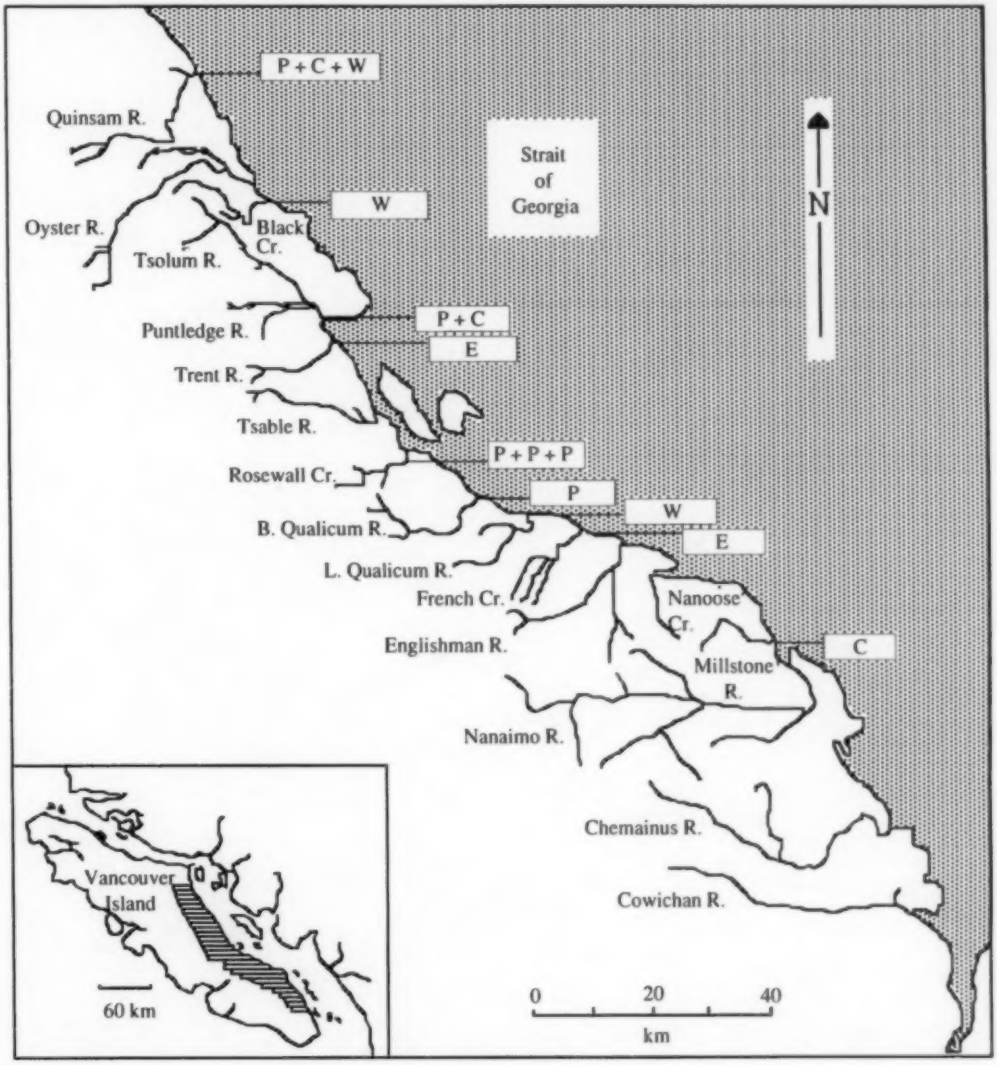
Fig. 1. Geographical location of study site. The abbreviations used to represent the stock types in each stream are production (P), colonization (C), enhanced (E), and wild (W).



(age 3+ and 4+, respectively), while jacks refer to those that mature in 10 months or less (age 2+).

Total recoveries by catch stratum were pooled across adjacent strata to eliminate those with negligible numbers and facilitate the comparisons of distribution patterns. Pooling was done in such a fashion as to minimize loss of information about latitudinal gradients in catch distribution, which are indicative of the migration range of each stock. The final catch strata and corresponding DFO statistical areas used for the analysis were north coast (sport, troll, and net fisheries of southeastern Alaska and areas 1–5), central coast (sport, troll and net fisheries of areas 6–11, plus sport and troll fisheries of areas 12 and 30), Johnstone Strait net (areas 12 and 13), northwestern Vancouver Island (troll and net fisheries of areas 25–27), Strait of Georgia troll (areas 13–18 and subareas of 29), northern Strait of Georgia sport (areas 13–16; freshwater catches included), Strait of Georgia sport (areas 17–20, 28, and 29; freshwater catches included), southwestern Vancouver Island (troll and net fisheries of areas 21–24 and sport fisheries of areas 21–27), Strait of Georgia net (areas 14–18 and 29), and South (Washington sport, net, and troll fisheries plus net fishery of area 20).

To compare survival rates of hatchery and nonhatchery releases, total recoveries in catch and escapement strata were adjusted slightly. For each stock subject to tagging during the smolt migration, total recoveries in all strata were expanded by a factor of 1.19 to account for the hypothesized differential mortality caused by trapping operations (see Blankenship and Hanratty 1990). Survival rates were then estimated from the ratio of total return to total release, i.e., the sum of total recoveries for jacks and adults in catch and escapement strata to the number of tagged smolts of the same stock and brood year. Adult exploitation rates were estimated from the ratio of total adult recoveries in the catch to total adult returns.

Effects of various factors on survival and exploitation

A linear logistic model (Fienberg 1980) was used to describe the

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Table 1. Attributes of stock and streams monitored during 1985–1988.

	Wild		Enhanced		Colonization		Production	
	Range	Median	Range	Median	Range	Median	Range	Median
Stream latitude	50–141	118	41–93	93	8–141	99	59–141	73
Hatchery rearing period	0–0	0	3–3	3	6–6	6	18–18	18
Smolt size at release	8.9–18.5	14.9	7.5–10.0	9.6	8.1–17.2	15.4	12.5–30.2	19.9
Smolt migration date	132–150	139	136–152	146	131–145	145	128–150	141
Smolt migration period	12–31	21	20–34	26	24–41	35	3–14	6
% adult catch outside	0.18–0.72	0.39	0.16–0.40	0.30	0.22–0.70	0.45	0.20–0.61	0.33
% jacks in total return	0.001–0.143	0.049	0.000–0.091	0.017	0.004–0.064	0.027	0.021–0.253	0.085
Adult run start	276–322	286	289–326	304	272–326	286	272–315	288
Adult run duration	3–9	7.5	3–7	5	3–10	9	5–10	9
Adult size	44.2–48.6	46.1	43.1–47.7	46.3	44.2–49.2	45.8	41.3–46.9	45.3
Total release	16 356–36 000	22 384	8 985–24 600	15 849	8 158–29 164	13 298	9 848–220 929	42 182
Total return	801–6 822	1 906	527–2 542	1 399	673–5 034	966	659–20 192	1 808
Adult escapement	95–1 541	413	58–626	173	29–1 305	164	39–5 394	191
Adult catch	559–3 721	1 004	378–1 998	990	559–3 375	914	455–13 078	1 595

Note: Adult escapement and catch figures are for age 3+ adults only. See text for description of variables.

relation between the tag recovery patterns and the factors monitored. The objective was to predict the probability of surviving to maturity, or being caught, for different types of coho characterized by combinations of population attributes and environmental conditions. The logistic model describing this relation is

$$(1) \qquad E\left(\frac{r_1}{r_2}\right) = \frac{e^{(b_0 + b_1x_1 + b_2x_2 + ..)}}{1 + e^{(b_0 + b_1x_1 + b_2x_2 + ..)}}$$

where r_1 is the total return (survival study), or total adult recoveries in the catch (exploitation study); r_2 is the total release (survival study), or total adult return (exploitation study); b_n is the parameter to be estimated; and x_n is the factor tested (x_1 is the year of return, x_2 is the smolt size, etc.)

The ratio of the frequencies in each category (r_1, r_2) is the response variable of interest. The factors for which information is available are as follows: (1) year of age 3+ adult escapement (or smolt migration for survival study); (2) parental genotype category (Quinsam River, Black Creek, Puntledge River, Trent River, Big Qualicum River, Little Qualicum River, French Creek, and Millstone River. Wild, colonization, and production coho residing together were assigned to the same category. Rosewall Creek releases were categorized according to the brood stock used); (3) release location, expressed as a latitudinal distance (km) from Nanaimo; (4) hatchery rearing period (0, 3, 6, and 18 months for wild, enhanced, colonization, and production, respectively); (5) smolt weight at release; (6) median smolt migration date; (7) smolt migration period (weeks); (8) fraction of age 3+ adults caught outside the Strait of Georgia; (9) jack contribution to total returns; (10) age 3+ run start date; (11) age 3+ run period (weeks); (12) mean fork length of age 3+ adults in escapement samples; (13) total release; and (14) total return (survival study) or total adult return (exploitation study). A summary of key population attributes that served for the present analyses is given in Table 1.

Since the number of effects (main + interactions) that could be evaluated exceeded the number of observations (35 stock–year combinations), it was necessary to limit the number and type of effects tested simultaneously. Main factors related to each other were identified by means of correlation matrices, and only one factor from each correlated pair ($r^2 > 0.50$) was allowed in the model. First-order interactions and main factors were tested only if some mechanism could be formulated to account for their influence. First-order interactions were allowed in the model if one of the two factors had been included previously. No higher order interactions were tested. Consequently, factors 7, 9, 11, 12, and 13 were not used for the survival analysis, and factors 7, 8, 9, 11, 13, and 14 were not used for the exploitation analysis. Note that if factor 14 is included in the model, it serves to compute the elements on both sides of eq. 1. This is not a desirable model property but was judged necessary to determine if the number of tag recoveries per se was large enough to provide consistent estimates of survival and account for their effects (if any).

The stepwise logistic regression program of the BMDP statistical package (Dixon et al. 1988) was used to fit the logistic model to the data. The fitting procedure was described by Labelle (1992) and followed that proposed by Green and Macdonald (1987). Essentially, the program adds or deletes factors in the model sequentially and, at each step, determines if the change leads to a significant improvement in fit. The model-building process was terminated when the reduction in deviance was less than 5% of the largest reduction in deviance obtained during previous steps. As a result, factors that had relatively small contributions might not be present in the model selected, even if such contributions were considered "statistically" significant. By omitting factors with lesser contributions, the model selected was more parsimonious with little loss in "predictive" ability. Once a model had been selected, insight into the effects of each factor was obtained by predicting survival or exploitation using combinations of parameter values selected within a realistic range. Thus, no extrapolations were made outside the data range.

Tests of similarity and covariation in survival and exploitation

The Shannon–Weaver diversity index (Zar 1984) was used as a measure of diversity in catch distribution. Indices were generated for each stock–year combination by setting the index variable p as the fraction of the total catch in each catch stratum. Pairwise Kolmogorov–Smirnov tests (Zar 1984) were used to assess the similarity levels between stocks in catch distributions. As recommended for this type of test, comparisons were based on the number of observed recoveries per stratum. These were adjusted proportionally to total 100 recoveries per stock–year combination to compensate for large differences in the number of recoveries between some stocks.

Statistical comparisons of exploitation patterns among stocks were hampered by the lack of replicates for all stocks within each year. Theoretical estimates of the variance associated with tag recoveries have been proposed for some fisheries in Alaska (Clark and Bernard 1987), but there is no consensus on the applicability of such estimates to commercial and sport fisheries in British Columbia. Therefore, exploitation rates were expressed as catch-to-escapement ratios of total recoveries and compared by means log-likelihood ratio tests (Zar 1984). Survival rates were expressed as ratios of total return to total releases minus total return (survivor:dead), and contrasted with the same statistical procedure. A probability level of 5% was

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used as a null hypothesis rejection criterion for establishing significant differences.

To determine the level of covariation in survival rates, changes in survival between consecutive years were calculated for each stock. Stocks monitored for 3 years were characterized by two point estimates (1986–1987 and 1987–1988) that act as coordinates in a two-dimensional plot. Stocks exhibiting comparable year-to-year changes in survival appear as clusters in such plots. Monte Carlo simulations were conducted to determine the clustering patterns that could have been obtained by chance alone. For each stock–year combination, random deviates were generated from a binomial distribution with a probability of success set to the mean annual survival rate. Differences between the simulated rates in consecutive years were considered as plot coordinates. The "expected" cluster size was determined from the number of stocks that fell within a cluster area comparable with the one based on empirical observations. This process was repeated 1000 times to produce a frequency distribution of expected cluster sizes. The same procedure was repeated for exploitation rates.

Results

Temporal and geographical trends in survival

Ocean survival estimates ranged from 0.5 to 23.1% (Fig. 2). Survival estimates averaged across stocks were 10.1, 8.0, and 9.6% for 1986–1988, respectively. No stock or stock type exhibited consistently greater survival, but the Big Qualicum production releases always had the lowest survival. For this stock, survival rates were much lower than the 15–42% reported by Mathews (1984) for 1975–1982, perhaps due to the hypothesized long term shifts in ocean conditions (Beamish 1995).

Smolts from various brood stocks released from Rosewall Creek exhibited similar survival. None of the stocks exhibited consistently higher or lower survival. However, each of these showed lower survival than their natural counterparts for both years. The differences averaged about 6.2 percentage points (PP) for the six stock–year comparisons. Differences tended to be greatest for Black Creek broods, and lowest for Little Qualicum River broods, suggesting an apparent relation between survival and the distance from the donor stream to the release location.

In nearly all cases where statistical comparisons were made between adjacent populations within a given year, significant differences in survival rates were detected. Differences in survival between stocks from the same stream averaged 4.4 PP over all possible comparisons (range 0–14), while those between stocks from adjacent streams averaged 7.7 PP (range 0.5–15.6).

Predicted effects of various factors on survival

Model 12 was identified as the most suitable predictor of survival rates (Table 2). The model provided a good fit to the data ($r^2 = 0.95$), and no aberrant pattern was detected in the residuals. The model fit was mainly influenced by genetic makeup, year, and the median smolt migration date. Hatchery rearing periods and stream latitude had smaller influences (Table 3).

Predicted survival was positively related to the proportion of total catch in outside waters, with a 25% increase in outside proportions translating into a comparable increase in predicted survival. Survival was also positively related to total returns, but doubling total returns increased the predicted survival by less than 0.25 PP for the groups tested. This suggests that survival estimates for stocks with small returns may be more in error than those of stocks with large returns. The magnitude of the effect was not large enough to cause serious concern about possible biases in survival or exploitation estimates, but this observation suggests that catch sampling regimes should be increased if monitoring activities are to focus on the status of weak or depleted stocks.

Predicted survival rates were inversely related to stream latitude and hatchery rearing period. The influence of smolt size on predicted survival was greatest when the peak migration date occurred relatively early in the season. Delays in smolt migration date always caused an increase in predicted survival. A 1-week delay in the ocean entry of Puntledge River colonization coho caused the predicted survival to increase by 1.3 PP one year and 1.7 PP the next. Small changes in time of ocean entry were found to have a greater effect on predicted survival than changes in size at release and hatchery rearing periods. These results agree with those of Bilton et al. (1982), who showed that release size had less effect on coho survival rates than release date.

The influence of genetic factors was assessed by predicting survival rates for different Rosewall Creek groups after adjusting for differences in smolt weight and total recoveries within each year. The predicted survival rates for 1986 and 1987 were, respectively, 5.0 and 5.4% for Black Creek broods, 9.7 and 10.4% for Trent River broods, and 8.1 and 8.7% for Little Qualicum River broods. Thus, if all smolts had been released at identical weights each year, smolts from Trent River brood would have exhibited consistently greater survival, while those of Black Creek brood would have exhibited the lowest. This conclusion would not have been reached based on a simple examination of the survival estimates for each group. This highlights the need to standardize time and size at release when trying to assess the effects of hatchery conditioning and genetic makeup on survival rates.

Covariation in survival

At most, four of the seven stocks monitored throughout the study period showed a tendency to cluster (Fig. 3). If all stocks had been monitored throughout the study period, the largest potential cluster would have been obtained if the Quinsam River wild and the two Rosewall Creek stocks had grouped with the four stock cluster. This cluster would have covered roughly a 2.5×4 PP spread in survival rates and would have included at most half of the stocks or five of the nine streams monitored. The observed and potential clusters were not composed entirely of stocks from the same stream, or of the same type, and stocks from geographically close streams did not exhibit a greater tendency to covary.

The Monte Carlo simulation results revealed that stocks exhibiting simple random variation in survival rates as expected from a binomially distributed variate centered around the mean annual survival rate would have shown similar levels of covariation (Fig. 4). If the assemblage monitored had included seven stocks, three of these would likely have clustered in the same area. In the 14-stock case, 5 of these would likely have clustered. This suggests that the pattern of covariation in survival observed within this group of stocks is similar to what would be expected from chance alone.

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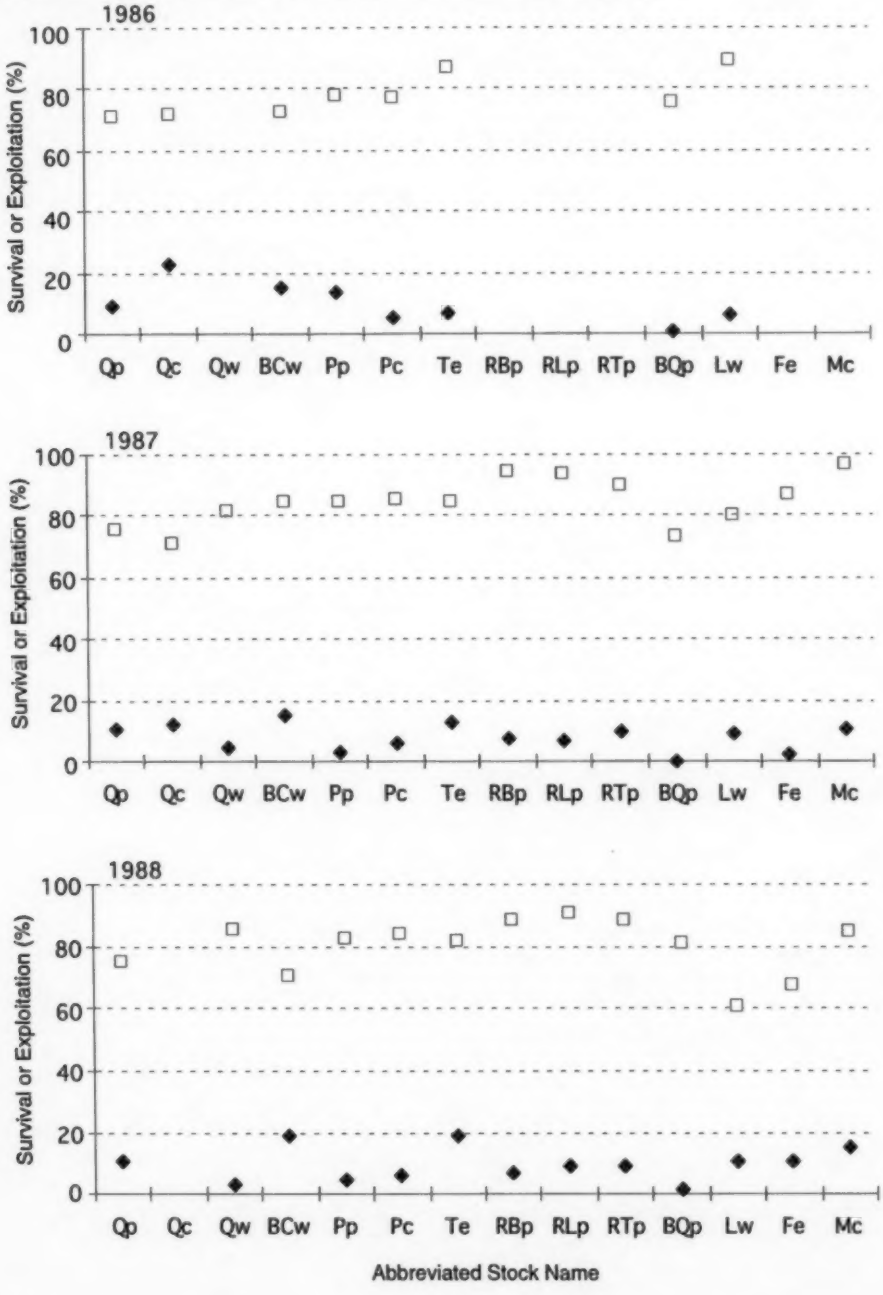
Fig. 2. Survival rates (solid diamonds) and exploitation rates (open squares) by age 3+ adult return year. Stock names are abbreviated in order from north to south: Quinsam River (Q); Black Creek (BC); Puntledge River (P); Trent River (T); Rosewall Creek releases of Black Creek, Little Qualicum, and Trent River broods (RB, RL, RT); Big Qualicum River (BQ); Little Qualicum River (L); French Creek (F); and Millstone River (M). Stock type abbreviations are production (p), colonization (c), enhanced (e) and wild (w).



Age composition in catches and total returns

The contribution of jacks to total returns ranged from 0 to 25.3%. Production stocks from the Quinsam, Puntledge, and Big Qualicum rivers had the largest proportions of jacks in total returns (mean 15%), while enhanced and colonization groups tended to have the lowest (mean 4%). For some stocks, the proportion of jacks in total returns varied considerably from year to year, but the magnitude and direction of the changes were not consistent across years.

Within each year, significant correlations were found

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Table 2. Composition of logistic models describing ocean survival and exploitation rates, with the corresponding degrees of freedom (df) and deviance (G).

	Main factors	Interactions	df	G
Survival				
Model 1	Constant (k)		34	75 879
Model 2	k, x_3		33	54 345
Model 3	k, x_3, x_4		32	44 521
Model 4	k, x_3, x_4, x_{14}		31	39 097
Model 5	k, x_3, x_4, x_{14}, x_2		24	15 185
Model 6	k, x_3, x_4, x_{14}, x_2, x_5		23	14 148
Model 7	k, x_3, x_4, x_{14}, x_2, x_5, x_8		22	11 574
Model 8	k, x_3, x_4, x_2, x_5, x_8	$x_5 \times x_6$	22	10 620
Model 9	k, x_3, x_4, x_2, x_5, x_8	$x_5 \times x_6$, $x_2 \times x_5$	15	6 035
Model 10	k, x_3, x_4, x_2, x_5, x_8	$x_5 \times x_6$, $x_2 \times x_3$, $x_1 \times x_6$	13	4 028
Model 11	k, x_3, x_4, x_2, x_5, x_8, x_1	$x_5 \times x_6$, $x_2 \times x_5$, $x_1 \times x_6$	11	2 800
Model 12	k, x_3, x_4, x_2, x_5, x_8, x_1, x_{14}	$x_5 \times x_6$, $x_2 \times x_5$, $x_1 \times x_6$	10	1 176
Model 13	k, x_3, x_4, x_2, x_5, x_8, x_1, x_{14}	$x_5 \times x_6$, $x_2 \times x_5$, $x_1 \times x_6$, $x_3 \times x_5$	9	530
Model 14	k, x_3, x_4, x_2, x_5, x_8, x_1, x_{14}, x_{10}	$x_5 \times x_6$, $x_2 \times x_5$, $x_1 \times x_6$, $x_3 \times x_5$	8	422
Exploitation				
Model 1	Constant (k)		34	3 180
Model 2	k, x_{12}		33	2 357
Model 3	k, x_{12}	$x_3 \times x_5$	32	2 016
Model 4	k, x_{12}, x_{10}	$x_3 \times x_5$	31	1 850
Model 5	k, x_{12}, x_{10}, x_4	$x_3 \times x_5$	30	1 626
Model 6	k, x_{12}, x_{10}, x_4, x_2	$x_3 \times x_5$	23	802
Model 7	k, x_{12}, x_{10}, x_4, x_2	$x_3 \times x_5$, $x_3 \times x_{10}$	22	680
Model 8	k, x_{12}, x_{10}, x_4, x_2, x_3	$x_3 \times x_5$, $x_3 \times x_{10}$	21	499
Model 9	k, x_{12}, x_{10}, x_4, x_2, x_3	$x_3 \times x_5$, $x_3 \times x_{10}$, $x_2 \times x_6$	14	213
Model 10	k, x_{12}, x_{10}, x_4, x_2, x_3, x_5	$x_3 \times x_5$, $x_3 \times x_{10}$, $x_2 \times x_6$	13	152
Model 11	k, x_{12}, x_{10}, x_4, x_2, x_3, x_5, x_1	$x_3 \times x_5$, $x_3 \times x_{10}$, $x_2 \times x_6$	12	135
Model 12	k, x_{10}, x_2, x_3, x_5, x_1	$x_3 \times x_5$, $x_3 \times x_{10}$, $x_2 \times x_6$, $x_4 \times x_5$	13	127

Note: The factor subscripts are (1) adult return year (exploitation study) or smolt migration year (survival study); (2) parental genotype; (3) stream latitude; (4) hatchery rearing period; (5) smolt weight at release; (6) median smolt migration date; (7) smolt migration period; (8) fraction of adults caught outside the Strait of Georgia; (9) jack contribution to total returns; (10) adult run start date; (11) adult run period; (12) mean adult fork length; (13) total release; (14) total return (survival study) or total adult return (exploitation study).

between the average weight of the smolts at release and the proportion of jacks in total returns ($0.54 < r^2 < 0.74$), as had been noted by Bilton et al. (1984). No relation was detected between the median date of juvenile migration and the proportion of jacks in total returns. Stocks from the Millstone, Big Qualicum, and Puntledge rivers tended to have higher proportions of age 4+ adults (mean 1%), but no stock or stock type had a consistently larger proportion of age 4+ adults in total returns. Tagged coho were recovered mainly as age 3+ fish in the Strait of Georgia sport fishery. Total catches of age 3+ coho often exceeded the corresponding escapements. Catches of jacks and age 4+ adults always accounted for <3% of total returns, so only those of age 3+ coho were used for comparing catch distribution patterns.

Temporal and geographical trends in catch distribution

There was a tendency, particularly during 1986, for northern stocks to contribute more to the central coast and Johnstone Strait net fisheries and for southern stocks to contribute more to the southern troll and the sport fisheries (Fig. 5, strata 2 and 3). This distinction was less pronounced in 1987–1988 with the increasing contribution of each stock to the sport fishery in the northern part of the Strait of Georgia (Fig. 5, stratum 6). The change in catch distribution from 1986 to 1988 could have been induced by a 14% increase of the sport fishing effort in the Strait of Georgia, that was accompanied by a 90% increase in sport catch. Suspected declines in chinook stocks (*Oncorhynchus tshawytscha*) may have contributed to the increase in coho catch in the Strait of Georgia through a redirection of fishing effort. An alternative hypothesis is that a greater proportion of coho remained in the Straight of Georgia and were thus more vulnerable to the sport fishery.

Net fishery openings in the Johnstone and Juan de Fuca straits occur intermittently during the migration periods of major salmon runs of southern British Columbia and Puget Sound. Coho are caught incidentally in these fisheries that target primarily on chum (*O. keta*), pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) stocks. The ratio of catches in the net fisheries of Juan de Fuca to those of Johnstone Strait was higher for southern stocks each year. Linear regressions of the catch ratios against the latitude of the stream of release showed a significant relation between both variables for 1986 and 1987 ($F = 23.4, 16.2$, $P < 0.003$). This relation was also evident but not significant during 1988. The year-to-year consistency in the trends are believed to reflect differences among stocks in the migration route followed on their return from outside

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Table 3. Relative influence of various factors on survival and exploitation rates as predicted from model 12 (Table 2).

	Factor omitted	Log likelihood	Chi-square	Difference	df	*P*
Survival						
M + I	None	–345 827.8	1 176.6	—	—	
M + I – x_2	Genotype	–350 350.0	10 330.0	9153.3	(7)	<0.001
M + I – x_1	Year	–347 163.0	3 956.0	2779.3	(2)	<0.001
M + I – x_{14}	Total return	–346 635.6	2 901.2	1724.6	(1)	<0.001
M + I – x_5	Smolt weight	–346 392.2	2 414.5	1237.8	(1)	<0.001
M + I – x_8	Proportion outside	–346 155.7	1 941.5	764.8	(1)	<0.001
M + I – x_3	Stream latitude	–345 958.1	1 546.2	369.6	(1)	<0.001
M + I – x_4	Hatchery rearing	–345 856.8	1 343.7	167.1	(1)	<0.001
M + I – $x_1 \times x_6$	Year × smolt migration date	–349 524.8	8 679.6	7503.0	(2)	<0.001
M + I – $x_2 \times x_5$	Genotype × smolt weight	–346 106.4	1 842.9	666.2	(7)	<0.001
M + I – $x_5 \times x_6$	Smolt weight × migration date	–345 827.8	1 285.6	109.0	(1)	<0.001
Exploitation						
M + I	None	–47 466.9	127.4	—	—	
M + I – x_2	Genotype	–47 718.6	631.8	504.4	(7)	<0.001
M + I – x_{10}	Start adult run	–47 689.2	573.1	445.7	(1)	<0.001
M + I – x_3	Stream latitude	–47 597.1	388.7	261.3	(1)	<0.001
M + I – x_5	Smolt weight	–47 498.9	192.4	65.0	(1)	<0.001
M + I – x_1	Year	–47 475.6	145.7	18.3	(1)	<0.001
M + I – $x_2 \times x_6$	Genotype × smolt migration date	–47 724.5	643.6	516.2	(7)	<0.001
M + I – $x_3 \times x_{10}$	Stream latitude × run timing	–47 619.1	432.7	305.3	(1)	<0.001
M + I – $x_3 \times x_5$	Stream latitude × smolt weight	–47 486.0	166.6	39.2	(1)	<0.001
M + I – $x_4 \times x_5$	Rearing period × smolt weight	–47 485.6	165.7	38.3	(1)	<0.001

Note: The model including all main and interaction effects is denoted by M + I. Goodness-of-fit tests were performed in the presence and absence of each factor. The factor removed precedes the minus sign, and the resulting difference in chi-square is given under Difference. Parenthetical df represent the degrees of freedom of the factor omitted.

waters. The decrease in average catch ratios from 1986 to 1988 appears to indicate a general reduction in the proportion of each stock returning through the southern route, but this trend is not clear given the annual variation in effort and its distribution within each region.

Coho stocks contributed to the Strait of Georgia net fisheries (stratum 10, Fig. 5) that target on large runs of chum salmon returning mainly to the Big Qualicum River hatchery. This fishery intercepted mainly stocks located in the center of the study area. The coho catch in this fishery usually accounted for a small fraction of the adult catch (mean 2%, range 0–13%). However, this fishery can have a pronounced impact on mature coho that previously escaped the troll, sport, and net fisheries. Judging from the ratio of net catches to the sum of escapement and net catches, this net fishery impacted primarily on spawners returning to Rosewall Creek.

The Kolmogorov–Smirnov tests of catch distribution patterns help identify assemblages of stocks with similar catch distributions (Table 4). An assemblage is defined here as a group of stocks from neighboring streams, characterized by the absence of significant difference ($P < 0.05$) in catch distribution between any two stocks in the assemblage.

For 1986, two assemblages emerge. The first one included the Puntledge River production stock and all others to the north. The second assemblage included all stocks south of this river for which catch data were available. The assemblages differed mainly in terms of their contribution to northern fisheries. Similar results were obtained for 1987, with the second assemblage contributing less to northern fisheries than the first. Note that the Little Qualicum stock is no longer included in the second assemblage because its catch distribution pattern differed from that of the Rosewall Creek production stock (RBp). The composition of each assemblage was also similar in 1988, except that the production stock from the Puntledge River is now included in the second assemblage.

The influence of the Strait of Georgia net fishery on catch distribution patterns was assessed by repeating the same tests using recovery data for only nine regions (omitting stratum 10). Nearly identical results were obtained, and the number and type of pairwise comparisons that resulted in significant differences remained the same. This suggests that this fishery was not a major determinant of the catch distribution patterns and the stock composition of each assemblage.

The consistent similarities observed over three seasons suggest that the location of a stream has some influence on the catch distribution of its coho population. The geographical boundary separating the northern and southern assemblages appears to lie in the vicinity of the Puntledge River. However, such boundaries are not fixed since the southern boundaries of the first and second assemblages moved during the study period. Note that, within each assemblage and even within a single river, significant differences in catch distributions were detected in some years. Significant differences in catch distribution patterns were also detected between stocks of adjacent streams not included in the two assemblages and between several stocks included in different assemblages.

Temporal and geographical trends in exploitation

Adult exploitation rates were 71–89% for 1986, 71–97% for 1987, and 61–91% for 1988 (Fig. 2). Annual exploitation rates, averaged across stocks, were 77.9, 84.8, and 80.3% for 1986–1988, respectively. The influence of the Strait of Georgia

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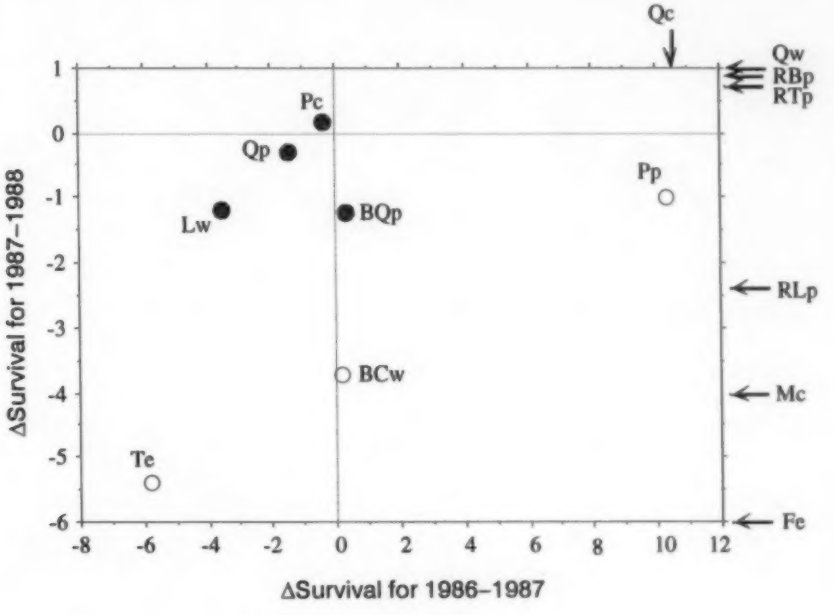
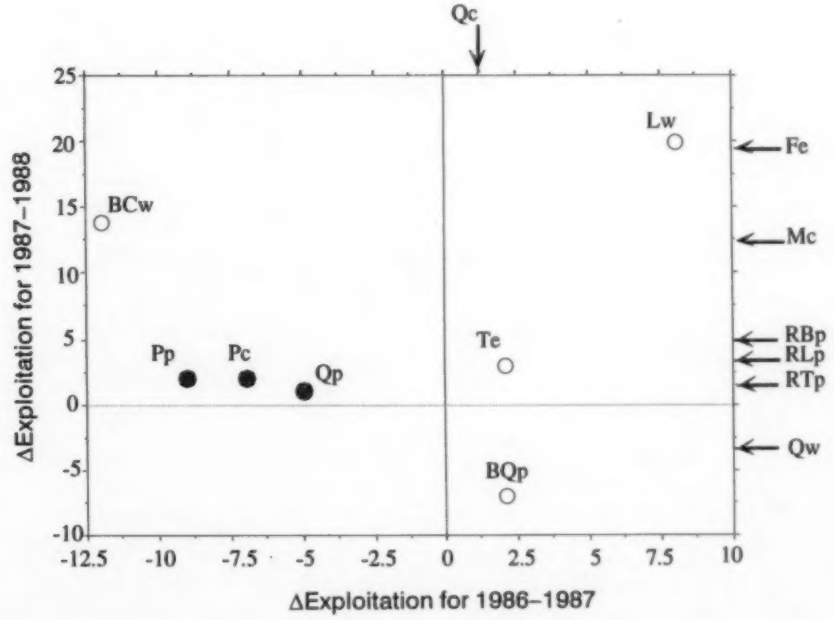
Fig. 3. Pattern of covariation in survival (top) and exploitation (bottom). The coordinates of each stock monitored for three consecutive years is expressed in terms of the differences in survival or exploitation between successive seasons (Δ in percentage points). Stocks monitored for only 2 years are lined along each axis at a level corresponding to the single difference. Shaded circles indicate cluster members. See Fig. 2 for abbreviations.





net fishery on exploitation rates was assessed by adding the net fishery catches to the corresponding escapements and then recalculating exploitation rates. In the absence of this fishery, the mean exploitation rates during the 3 years would have decreased by 3.5, 4.7, and 1.4 PP, respectively, so this fishery had a relatively small influence on the overall exploitation rates.

No stock or stock type was subject to consistently low or high exploitation in all years. Exploitation rates of colonization

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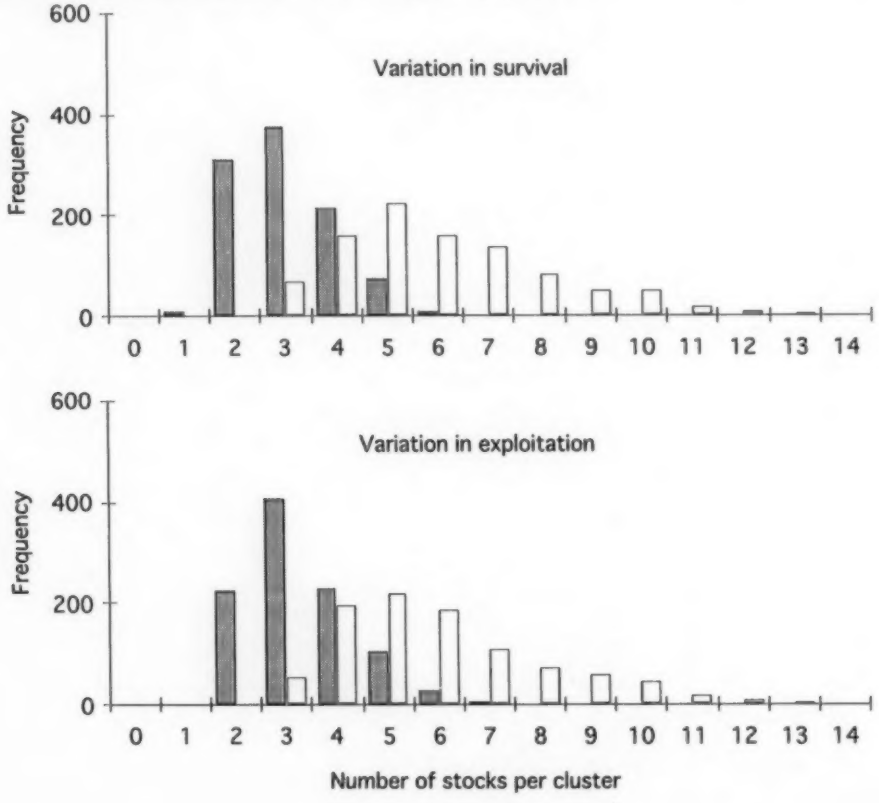
Fig. 4. Results of numerical simulations of clustering patterns. Top histogram represents the expected number of stocks that would cluster in a 2.5 × 4 percentage point area if survival rates exhibited random variation as expected from a binomially distributed variate centered on the mean annual survival rates. Shaded and open bars represent the distribution of outcomes for 7 and 14 stocks, respectively. Lower histogram indicates the expected number of stocks that would cluster in a 5 × 5 percentage point area.



and production stocks from the same river never differed by more than 5 PP (usually <2 PP from each other). However, exploitation rates on wild coho from the Quinsam River were always higher (7–11 PP) than for production and colonization coho from this river. The difference could not be attributed solely to any single smolt characteristic, because wild and production smolts differed in migration pattern, size, and hatchery rearing periods.

Exploitation rates on production releases from the Rosewall Creek hatchery never differed by more than 4 PP, and none of the three groups released from this hatchery was subject to consistently larger or smaller exploitation rates. This suggests that exploitation rates on different strains released from the same hatchery can be similar when brood stock is obtained from other streams located within 45 km from the rearing and release site. Exploitation rates on stocks released from Rosewall Creek were always 5–35 PP greater than those on the same stocks reared naturally. Thus, coho stocks reared and released from a hatchery located on a nonparental stream can be subject to exploitation rates that differ from their natural siblings, even when their respective smolts populations migrate to sea at similar times and sizes.

Linear regressions of diversity scores against the number of tags released and against the number of tags recovered revealed no significant relation in any year. Linear regressions revealed no significant relation between the diversity scores and exploitation rates in any year, and no stock exhibited consistently higher or lower indices of diversity across all years. However, the groups released from Rosewall Creek had consistently higher scores ($0.89 < D < 0.91$), and those from the Millstone and Trent rivers had the next highest ($0.85 < D < 0.91$). Since these groups were the only ones exposed to groundwater and stream water during their rearing period, it is hypothesized that such rearing practices can affect the behavior of coho after release, perhaps by increasing their tendency to explore new environments.

Linear regressions revealed no significant relation between diversity scores and stream location across years. Within each year, stocks with later median smolt migration dates tended to have lower diversity scores, but no significant relations were detected between these variables. Still, this last observation supports the observations of Mathews and Ishida (1989), who noted a geographical compression of the catch distribution of coho stocks with relatively late smolt migration periods.

Predicted effects of various factors on exploitation

Model 12 was identified as the most suitable predictor of exploitation rates (Table 2). It provided a better fit to the data

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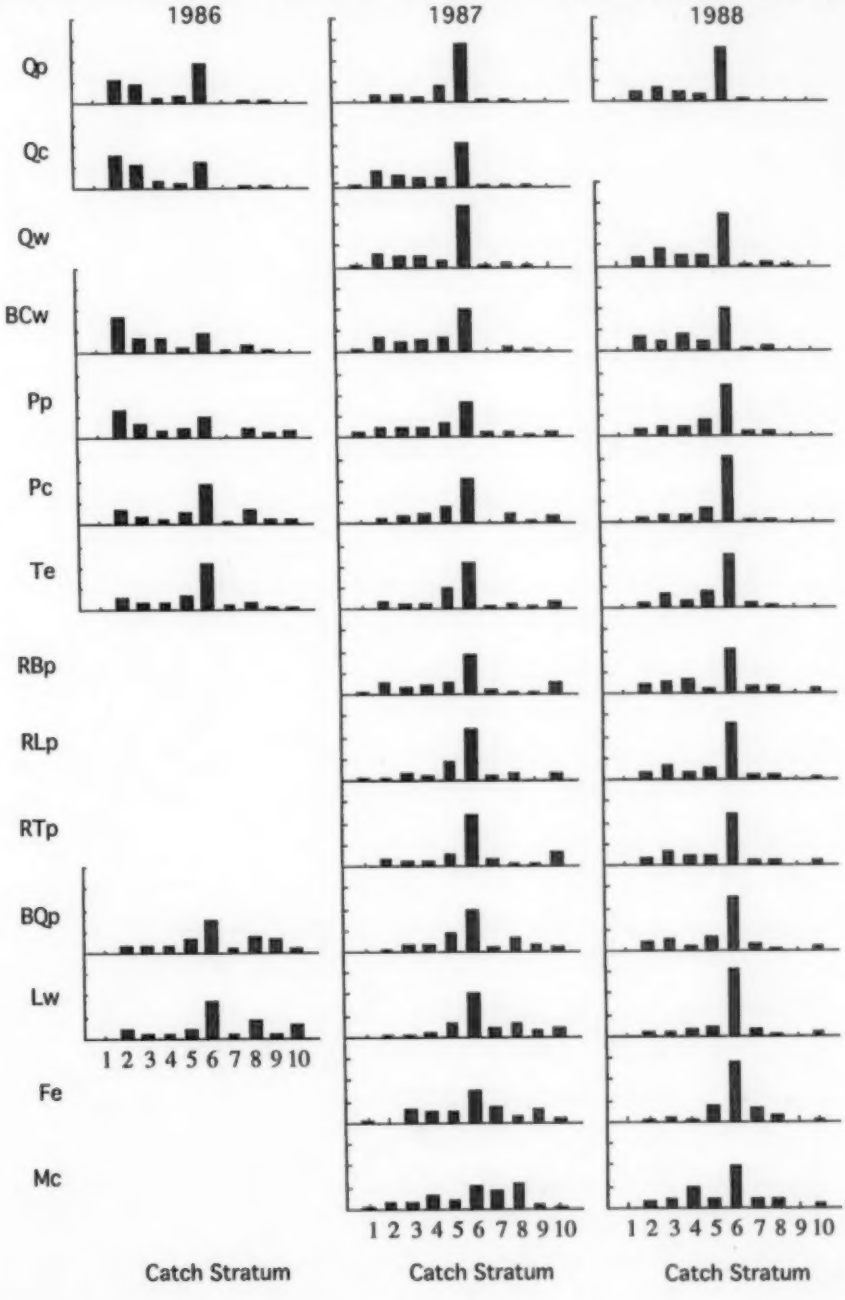
Fig. 5. Catch distribution patterns for age 3+ coho by stock and return year. The catch strata categories are ordered from north to south: north coast (1), central coast (2), Johnstone Strait net (3), northwestern Vancouver Island (4), Strait of Georgia troll (5), northern Strait of Georgia sport (6), southern Strait of Georgia sport (7), southwestern Vancouver Island (8), south (9), and Strait of Georgia net (10).



($r^2 = 0.95$) than model 10 for the same degrees of freedom, and no aberrant pattern was detected in the residuals. The model fit was mainly influenced by the genotype, run timing, stream latitude, and smolt migration time (Table 3). Stream latitude and its interaction with run timing had a larger influence on exploitation than year and smolt weight.

Delays in smolts migration caused an increase in predicted exploitation rates on stocks from the Big and Little Qualicum rivers. The latter showed a more pronounced increase for comparable increases in release time (genetic × migration date interaction). This suggests that the relatively low exploitation rates on the Big Qualicum stock during 1987 could have been

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Table 4. Results of comparisons of catch distributions using Kolmogorov–Smirnov tests.

	Quins (p)	Quins (c)	Quins (w)	Black (w)	Puntl. (p)	Puntl. (c)	Trent (e)	RB (p)	RL (p)	RT (p)	B. Qual (p)	L. Qual (w)	French (c)
1986													
Quins. (p)	·												
Quins. (c)	0.116	·											
Quins. (w)	—	—	·										
Black (w)	0.242	0.986	—	·									
Puntl. (p)	0.223	0.111	—	0.240	·								
Puntl. (c)	0.011	0.000	—	0.000	0.016	·							
Trent (e)	0.000	0.000	—	0.000	0.001	0.988	·						
Rosew. (BC)	—	—	—	—	—	—	—	·					
Rosew. (LQ)	—	—	—	—	—	—	—	—	·				
Rosew. (TR)	—	—	—	—	—	—	—	—	—	·			
B. Qual. (p)	0.001	0.000	—	0.000	0.002	0.468	0.200	—	—	—	·		
L. Qual. (w)	0.000	0.000	—	0.000	0.000	0.263	0.099	—	—	—	0.805	·	·
French (e)	—	—	—	—	—	—	—	—	—	—	—	—	
Millst. (c)	—	—	—	—	—	—	—	—	—	—	—	—	—
1987													
Quins. (p)	·												
Quins. (c)	0.238	·											
Quins. (w)	0.787	0.722	·										
Black (w)	0.897	0.946	0.840	·									
Puntl. (p)	0.845	0.224	0.082	0.292	·								
Puntl. (c)	0.367	0.005	0.032	0.052	0.285	·							
Trent (e)	0.229	0.001	0.031	0.011	0.163	1.000	·						
Rosew. (BC)	0.699	0.181	0.069	0.267	0.818	0.813	0.404	·					
Rosew. (LQ)	0.211	0.001	0.038	0.015	0.113	1.000	1.000	0.468	·				
Rosew. (TR)	0.012	0.000	0.002	0.000	0.005	0.623	0.626	0.175	0.923	·			
B. Qual. (p)	0.145	0.000	0.007	0.006	0.104	1.000	1.000	0.279	0.998	0.555	·		
L. Qual. (w)	0.004	0.000	0.000	0.000	0.002	0.368	0.321	0.026	0.272	0.988	0.379	·	
French (e)	0.005	0.000	0.000	0.000	0.144	0.312	0.196	0.173	0.173	0.581	0.632	0.581	·
Millst. (c)	0.003	0.000	0.000	0.000	0.118	0.264	0.163	0.141	0.142	0.153	0.560	0.077	0.275
1988													
Quins. (p)	·												
Quins. (c)	—	·											
Quins. (w)	1.000	—	·										
Black (w)	0.996	—	0.988	·									
Puntl. (p)	0.083	—	0.182	0.037	·								
Puntl. (c)	0.002	—	0.006	0.001	0.766	·							
Trent (e)	0.220	—	0.415	0.110	1.000	0.510	·						
Rosew. (BC)	0.363	—	0.367	0.120	0.650	0.054	0.691	·					
Rosew. (LQ)	0.480	—	0.754	0.274	0.593	0.173	0.970	0.899	·				
Rosew. (TR)	0.427	—	0.692	0.179	0.981	0.267	0.974	1.000	1.000	·			
B. Qual. (p)	0.158	—	0.323	0.078	0.935	0.441	0.996	0.859	1.000	1.000	·		
L. Qual. (w)	0.001	—	0.005	0.000	0.057	0.755	0.039	0.044	0.082	0.179	0.281	·	
French (e)	0.000	—	0.000	0.000	0.040	0.485	0.017	0.000	0.004	0.005	0.028	0.749	·
Millst. (c)	0.208	—	0.211	0.295	0.529	0.037	0.460	1.000	0.689	0.994	0.754	0.030	0.000

Note: Values are the probabilities of pairwise test results. Shaded areas identify assemblages with similar catch distributions (all $P > 0.05$). p, production; c, colonization; e, enhanced; w, wild; BC, Black Creek; LQ, Little Qualicum River; TR, Trent River; —, stocks for which no catch distribution data were available.

induced by hatchery practices. Smolts were released from that hatchery relatively early during 1987, but if they had been released 12 days later at a slightly smaller size (15 g), the predicted exploitation rate would have been higher and close to the overall mean exploitation rate that year.

Colonization (or reducing hatchery rearing from 18 to 6 months) caused the predicted exploitation rates on the Big Qualicum stock in 1987 to decrease from 73 to 68% for similar time and size at release. However, headwater rearing environments are usually less conducive to growth than hatchery rearing ponds, so colonization smolts tend to migrate to sea later and at a smaller size than production smolts from the same system. Thus, changes in hatchery exposure could be more realistically assessed by accounting for the associated changes in time and size at release. Assuming a hatchery rearing period of 6 months, a 1-week delay in smolt migration date, and a release size of 15 g, the predicted exploitation rate would have decreased by about 2 PP. This illustrates how the influence of

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hatchery rearing time can be counteracted by the effects of time and size at release and shows the difficulty of detecting the effects of different hatchery rearing periods.

Delays in the upstream migration of adults resulted in higher exploitation, with a 10-day delay in run timing causing the predicted exploitation of the Big Qualicum stock to increase from 73 to 83%. How run timing influences exploitation is unclear. Delays in the first freshet may induce coho to stray or be subject to high mortality in the stream mouth owing to physiological stress or predation by seals. These processes would cause lower escapements and seemingly higher exploitation rates. Coho that enter the stream early in the season may also be less vulnerable to Strait of Georgia net fishery and to capture by saltwater anglers fishing near the stream mouths.

During 1988, the exploitation rate on the Big Qualicum River stock exceeded that of the Little Qualicum River stock (81 vs. 61%). In terms of time and size at release, Big Qualicum hatchery smolts emigrated 9 days earlier and were 6 g heavier than their counterparts. Reducing smolt weight by 6 g lead to an increase in the predicted exploitation rate on Big Qualicum River stock from 81 to 85%, while a 9-day delay in time of release increased it from 81 to 82.5%. Thus, differences in time and size at release alone do not account for all discrepancies in exploitation rates between these adjacent stocks. The Big Qualicum River run started about 2 weeks later than at Little Qualicum River. A 2-week delay would have caused the predicted exploitation rate to decrease from 81 to 66%. Thus, most of the discrepancy in exploitation between these stocks could be attributed to differences in run timing.

Interstock differences in exploitation

Significant differences in catch-to-escapement ratios were detected between certain stocks from adjacent streams within each year. Differences in exploitation rates between stocks from adjacent streams in the same year reached 20 PP and largely exceeded those between stocks from the same stream (usually <5 PP). Significant differences in catch-to-escapement ratios were also detected between certain stocks from the same assemblage within each year. Stock-specific exploitation rates differed by as much as 21 PP during 1987 in the southern assemblage and 18 PP during 1988 in the northern one. Consequently, the null hypothesis of no difference in susceptibility to exploitation among stocks was rejected.

Covariation in exploitation

Three of the seven stocks monitored throughout the study period appeared to cluster (Fig. 3). The remaining ones were scattered and failed to show much similarity. Stocks monitored for only 2 years could not be positioned in the plot but were lined up along one axis to indicate where they might have been located. The largest potential cluster would have been obtained if all Rosewall Creek stocks had grouped with the Puntledge–Quinsam stock cluster. This potential cluster of six stocks would have covered roughly a 5 × 5 PP spread in exploitation rates and would have included less than half of the stocks or three of the nine streams monitored. The observed and potential clusters were not composed entirely of stocks from the same stream, or of the same type, and stocks from geographically close streams did not exhibit a greater tendency to covary. The observed and potential clusters included stocks from different assemblages as defined earlier, and their stock compositions differed from those of the survival clusters.

The Monte Carlo simulation results revealed that stocks exhibiting simple random variation in exploitation rates as expected from a binomially distributed variate centered on the mean annual exploitation rates would have shown similar levels of covariation (Fig. 4). If the group had comprised seven stocks, it is likely that three of these would had exhibited covariation patterns as described above. In the 14-stock case, 5 of these would most likely clustered in the same area. Thus, the covariance pattern observed during this study was similar to what could have been expected from chance alone. However, the patterns observed do not support the notion that any particular stock is representative of the year-to-year variation in exploitation and survival rates among stocks within each assemblage, among stocks of the same type, or among stocks from a particular stream.

Discussion

Previous studies aimed at identifying the effects of environmental and biological factors upon ocean survival of coho salmon have primarily focused on the effects of single factors. This empirical investigation presented an opportunity to assess the influence of several factors operating simultaneously. Our analysis highlighted the difficulty of quantifying the influence of specific factors since their levels can be interconnected in uncertain fashions. This must be accounted for with some difficulty so that, when the level of one factor is tested for, the levels of related factors are allowed to vary in a commensurate fashion. For this reason, it is usually desirable to conduct such analyses on data sets that cover a broad spectrum of environmental conditions and population attributes. Our experimental design initially included more than 14 stocks and 9 streams and was more balanced in terms of the number of stock types and distribution. Unfortunately, financial and operational constraints prevented us from monitoring the additional stocks. Despite the limitations of this investigation, considerable insight was gained on the nature of the factors responsible for variation in survival and exploitation, which may be used to formulate more elaborate hypotheses for subsequent testing. Furthermore, the results obtained are, by and large, in agreement with those of other studies.

With regards to ocean survival, Bilton et al. (1982) showed that factors such as size at release have systematic effects upon survival: survival increases with smolt weight up to a certain point and then decreases afterwards with further increases in weight. Since the range of smolt sizes observed in the present study was much smaller than that tested by Bilton et al., there was no reason to assume the existence of a nonlinear relation between size and survival. Interestingly, increasing size at release did not always cause an increase in predicted survival, as noted by Mathews and Ishida (1989).

Mathews and Ishida (1989) reported that higher survival was always associated with later release dates. Bilton et al. (1984) conducted a series of smolt releases from the Quinsam River hatchery and found that, when smolts of similar weights were released at different intervals between late April and mid-June, survival increased up to late May and decreased thereafter. In the present study, all median dates of smolt migration

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occurred during May, which may explain why survival was always positively related to release date.

Bilton et al. (1982) suggested the existence of "optimum release windows" that provide optimal conditions for survival through the abundance of forage organisms. Mathews and Ishida (1989) found little evidence that coho released earlier encountered poor feeding conditions but suggested that predation was the major determinant of time-varying mortality. Bilton et al. (1982) noted that the relations between adult returns, release time, and release size (response surfaces) for the Rosewall Creek releases did not appear to be applicable to other hatcheries and suggested that other streams may have different optimum release windows.

Mathews and Ishida (1989) suggested that the optimum release date may in fact be an "interannually moving target" dependent upon a variable receiving environmen, and (or) a function of a smolting response that depends on variable freshwater rearing conditions. If the optimum release date does change from year to year, then one would expect that the year × migration date interaction would have a major influence upon survival, as was shown in the present study. Therefore, our results add some support for the interannually moving target hypothesis.

The influence of the outside rearing proportions on survival was somewhat anticipated. Mathews and Buckley (1976) noted that, among Puget Sound coho stocks, those that reared in the sound during their ocean life showed a more pronounced decline in abundance than the ocean-going populations. The authors suggested that more favorable ocean conditions exist in outside waters. Coho populations from tributaries to the Strait of Georgia are known to migrate to outside waters in different proportions (Argue et al. 1983). Ricker (1972) noted that there were consistent difference in mean size of coho taken in Puget Sound and the Strait of Georgia, as opposed to outside waters, and attributed such findings to environmental effects. After further analysis, Ricker (1981) concluded that stocks that migrated to outside waters grew faster than those remaining inside. Conditions favorable for growth may also be conducive to greater survival, which may explain why stocks migrating to outside waters tend to survive better than those remaining inside.

The results of this study suggest that the effects of genetic makeup on survival can outweigh those of hatchery conditioning. There is considerable evidence of the role of hereditary factors in characterizing life history traits of salmon populations (Ricker 1972). Certain coho strains are known to perform better than others under hatchery conditions. Gowan and McNeil (1984) gave evidence that coho smolts obtained from particular hybrid stocks can have higher ocean survival rates than other hybrid and nonhybrid coastal stocks. Our results suggest that progeny from the same parent stock should be used when comparing the performance of hatchery and nonhatchery stocks.

Various studies have shown that hatchery practices can influence subsequent fishery contributions (Bilton et al. 1982, 1984). The present study confirms that time and size at release can influence subsequent exploitation, but other factors can also have large influences. Production releases were observed to have similar catch distributions as their natural counterparts, but the former were generally subject to higher exploitation. This suggests that hatchery conditioning may make coho more vulnerable to fishing gear. Myers (1980) observed that hatchery smolts consumed a larger variety of items in the estuary than their wild counterparts. Perhaps hatchery coho acquire and maintain a tendency to feed less discriminantly than wild coho, and as a result, are more readily caught by hook and line gear in later life.

Our results indicate that stream location can have an important influence on exploitation patterns, as recently noted by Irvine and Bailey (1993). The first assemblage defined on the basis of fishery contribution included stocks of wild, colonization, and hatchery origin from a geographical region covering 23 km of coastline. Since no stocks located beyond the Quinsam River were monitored during this study, the northern boundary of this assemblage might extend further north. The second assemblage covered a coastal region of about 45 km and was also characterized by a mixture of stock types and an oscillating boundary. The fact that certain stocks were consistently included in each assemblage suggests some year-to-year stability in the assemblage composition and limits to the geographical extension of stock assemblages.

If one assumes that the exploitation patterns reflect the marine distribution of adults, then it appears that the stocks north and south of the Trent River follow different migration patterns. Little is known about the factors that control migration patterns, but it is hypothesized that adult distribution is linked to the juvenile migration patterns followed during the early marine stages (Hartt 1980; Pearcy and Fisher 1988). Pearcy and Fisher (1988) gave evidence that juvenile coho salmon usually remain in surface waters (0–20 m) and that their distribution may be largely affected by surface currents during early marine life. Healey (1980) hypothesized that coho that leave the Strait of Georgia might emigrate soon after ocean entry.

Relatively little is known about the surface currents in the Strait of Georgia (Leblond 1983). Based on the results of drift studies conducted since 1920, Thomson (1981) hypothesized that there are a northern and a southern counterclockwise gyre inside the Strait, which connect at about the latitude of the Puntledge River. Depending on the location of a stream, a portion of the smolts leaving each stream could get entrained in different gyres. Smolts from streams north of Puntledge River might be transported northward through Johnstone Strait, while others would move outside though the Strait of Juan de Fuca. Godfrey et al. (1975) noted that coho from Washington and southern British Columbia do not generally undertake long ocean migrations and tend to remain near rich feeding grounds until the onset of maturity. Once this stage is reached, a major portion of the harvest has been achieved, so fishery contributions are to a large extent determined by the distribution of coho prior to the spawning migration. Thus, it is hypothesized that circulation patterns inside the Strait of Georgia have some influence on the geographic boundaries of the assemblages and the level of similarity in fishery contribution among stocks in each assemblage.

Attempts to quantify the level of covariation in survival and exploitation rates were hampered by the unavailability of estimates for some stock–year combinations and short time series of data. Still, the results do not indicate a high level of similarity in survival and exploitation among stock types or among neighboring stocks. This is not all that surprising given the number of factors that can affect exploitation and survival. Predicted exploitation rates for instance were shown to be

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influenced by hatchery practices and run timing. Labelle (1990*b*) detected significant differences in run timing in some years among stocks from streams lacking flow control (nonhatchery systems) perhaps because their stream flows responded differently to weather conditions early in the season. Hatchery practices were not uniform across all hatchery systems and probably never will be, owing to the effects of disease outbreaks, broodstock availability, selection practices, and operational constraints. One should expect some unsynchronized year-to-year variation in run timing, time and size at release, and hatchery rearing conditions. Added to this are the effects of the Strait of Georgia net fisheries, which can have variable impacts on specific stocks. The end result is a situation characterized by much stochasticity in terms of escapement, survival, and exploitation patterns. Thus, it is hypothesized that longer time series of data would not reveal a high level of covariation in survival and exploitation rates among stocks within this area.

Should the above hypothesis be true, one would expect stock assemblages to show only weakly correlated responses to changes in marine conditions and fishing effort, particularly if such assemblages cover a large area and include stocks of various types and origin. If stocks of given types fail to show strongly correlated responses, then there appears to be little need to consider hatchery or nonhatchery stocks as separate entities, at least from an indexing point of view (see review by Waldichuk 1984). Fishery managers who assume that the exploitation or survival rates of stocks in one region are similar to those of an indicator stock should take caution. Based on our results, there seems to be no justification for assuming that any stock or stock type can be used as a yardstick for measuring the response of neighboring stocks to ocean conditions and fishing pressures with a high degree of reliability. At best, it appears that indicator stocks could be used to provide a crude idea of trends in mortality rates operating on stocks from a given region.

Recent assessments of the current status of coho salmon stocks in southern British Columbia indicated that escapements to natural streams have declined by 60–95% throughout the Strait of Georgia since the 1970s (Anonymous 1990). Exploitation rates on the stocks monitored for this study averaged about 80%, and in some instances reached up to 96%. Based on the stock–recruitment data, the maximum sustainable exploitation rate on natural coho stocks in Oregon was estimated to be 73% (ODFW 1981). Wong (1982) analyzed catch and escapement statistics obtained during the 1950–1975 period for stock assemblages from several coastal regions in British Columbia and estimated that the optimum harvest rate on natural coho stocks from the Strait of Georgia was 71%.

Given such facts, the 1986–1988 exploitation rates are considered to be relatively high for natural stocks. If sustained on a long-term basis, such high rates of exploitation could reduce the abundance of natural stocks to levels that are insufficient for maintaining genetic viability. In view of this, managers may want to reduce exploitation rates on wild stocks but maintain high rates on hatchery stocks to please fishermen. However, the hatchery stocks monitored during this study did not show distinct temporal or spatial patterns of marine distributions. As a result, it might prove extremely difficult to harvest hatchery and wild fish differentially through the traditional imposition of time, gear, and area closures.

Should fishery managers wish to adopt a precautionary approach (Anonymous 1995) for the management and exploitation of coho stocks, fishing plans would have to be designed to minimize the risk of exceeding the maximum sustainable exploitation rate. If indicator stocks are relied upon to meet this objective, then the level of exploitation on the indicator stock should be lower than the sustainable rate, since other stocks in the assemblage may be subject to higher exploitation than the indicator stock.

During the present study, differences in exploitation rates between the Quinsam River production stock and others within the northern assemblage ranged from –5 to 10 PP during 1987 and 1988. To ensure that exploitation rates on all stocks in the northern assemblage did not exceed the rate suggested by Wong (1982), the 1987 and 1988 exploitation rates on this indicator stock should have been about 60% or roughly 20 PP lower than they were. While this example serves to describe one possible application of the indicator stock approach, it does not provide much guidance for selecting an indicator stock and establishing the geographical boundaries of possible assemblages in other regions. It is hoped that long-term stock monitoring programs will be maintained and implemented in other regions to address this issue and help determine how variation in survival and exploitation is affected by climatic changes, geographic location, and fishing regime.

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Mitochondrial DNA evidence of distinct glacial refugia for brown bullhead (*Ameiurus nebulosus*) in the Great Lakes

Mary H. Murdoch and Paul D.N. Hebert

Abstract: Restriction fragment length polymorphisms were used to survey the mitochondrial genome of *Ameiurus nebulosus* for nucleotide sequence variation. Two hundred and forty-nine individuals were analyzed from 12 populations across the Great Lakes drainage and 3 populations in possible refugial drainages. Fifteen restriction endonucleases revealed 50 distinct haplotypes among these fish. Two major phylogenetic assemblages, A and B, were revealed with an average 3.22% sequence divergence. Both assemblages were themselves fragmented into two groups. Strong geographic patterning was observed in the frequency of assemblages and groups across the sampling area: assemblage A was predominant in fish from populations east of Lake Erie, while western populations were dominated by assemblage B. The distribution and phylogenetic divergence of mitochondrial haplotypes indicate that brown bullhead in the Great Lakes originated from two Pleistocene refugia and further provides genetic evidence of subdivision within these refugia. Pleistocene glaciations appear to have had a similar influence on the geographic distribution of mitochondrial DNA lineages of other North American fishes. In contrast with these other species, however, much of the mitochondrial DNA variation and divergence was retained in brown bullhead populations living south of the ice sheets and is represented in extant populations.

Résumé : Les polymorphismes de longueur des fragments de restriction ont été utilisés pour examiner le génome mitochondrial de *A. nebulosus* afin de déceler des variations de la séquence nucléotidique. On a analysé 249 sujets provenant de douze populations réparties dans le bassin hydrographique des Grands Lacs et de trois populations vivant dans des bassins refuges possibles. Quinze endonucléases de restriction ont révélé la présence de cinquante haplotypes distincts chez ces poissons. Deux grands assemblages phylogénétiques, A et B, ont été observés, la divergence de séquences moyennes étant de 3,22. Les deux assemblages étaient eux-mêmes fragmentés en deux groupes. Une forte caractérisation géographique a été observée dans la fréquence des assemblages et des groupes dans l'ensemble de la région échantillonnée : l'assemblage A a prédominant chez les poissons provenant des populations vivant à l'est du lac Érié, alors que les populations vivant à l'ouest étaient dominées par l'assemblage B. La distribution et la divergence phylogénétique des haplotypes mitochondriaux indiquent que les génétiques de subdivision à l'intérieur de ces refuges. Les glaciations du pléistocène semblent avoir eu un semblable sur la répartition géographique des lignées d'ADNmt d'autres poissons d'Amérique du Nord. Toutefois, à ces autres espèces, une bonne partie de la variation et de la divergence de l'ADNmt a été dans les populations actuelles.
[Traduit par la Rédaction]

Introduction

The Pleistocene glaciations had a profound impact on the distribution of North American freshwater fish. Each of the four major glacial advances (Nebraskan, Kansan, Illinoian, Wisconsinan) altered the climate over the entire continent and restricted northern fish to one or more of a series of ice-free refugia (Crossman and McAllister 1986). This long-term isolation undoubtedly led to morphological and genetic differentiation of populations as a result of mutation, natural selection, and genetic drift (Avise et al. 1987), providing markers of historical isolation among present-day populations. A number of studies have used distributional information and geographic patterns in morphology to postulate routes employed by fish taxa in their recolonization of the glaciated portions of North America (Bailey and Smith 1981; Crossman and McAllister 1986; Lindsey and McPhail 1986; Schmidt 1986; Underhill 1986; Mandrak and Crossman 1992). Over the past 20 years it has become possible to test the validity of these hypotheses via genetic analysis. For example, geographic patterning in allozyme frequencies has provided evidence of multiple glacial refugia for lake whitefish (*Coregonus clupeaformis*; Franzin and Clayton 1977), northern pike (*Esox lucius*; Seeb et al. 1987) and yellow perch (*Perca flavescens*; Todd and Hatcher 1993).

The most powerful technique now available to examine postglacial dispersal hypotheses is mitochondrial DNA (mtDNA) analysis. Genetic variation within and among conspecific populations arises more quickly in the mitochondrial than nuclear genome due to its more rapid rate of nucleotide substitution, its maternal mode of inheritance, and its lack of recombination (Moritz et al. 1987). Two major patterns of mtDNA diversity have been identified in cold-tolerant North American fish populations that are consistent with recent glacial impacts. Firstly, low genetic diversity is observed in fish

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Table 1. Sample locations (see Fig. 3) of brown bullhead populations, their drainage areas, sample size and number of haplotypes detected for each population, and genetic diversity indices of the populations (haplotype diversity and nucleotide diversity).

Code	Sample location	Sample size	No. of haplotypes	Haplotype diversity (h)	Nucleotide diversity ($d \times 100$)	Drainage
a	Shogomoc Lake, New Brunswick	26	2	0.5077	0.15	St. John River
b	Mica Lake, Ontario	20	2	0.1	0.01	Upper Rideau Lakes
c	Cape Vincent, New York	13	11	0.9744	0.54	Lake Ontario
d	Hamilton Harbour, Ontario	20	6	0.7895	2.23	Lake Ontario
e	Ashtabula Bay, Ohio	21	7	0.8286	1.93	Lake Erie
f	Cuyahoga River, Ohio	11	1	0	0	Lake Erie
g	Black River, Ohio	25	4	0.23	0.04	Lake Erie
h	Old Woman Creek, Ohio	12	8	0.8485	0.27	Lake Erie
i	Huron River, Ohio	25	8	0.6867	0.47	Lake Erie
j	Gibraltar Bay, Michigan	24	5	0.6594	0.74	Detroit River, Lake Erie
k	Point Pelee, Ontario	15	9	0.9243	1.15	Lake Erie
l	Lake St. Clair, Ontario	9	4	0.75	0.15	Lake St. Clair
m	Des Plaines River, Illinois	4	3	0.8333	0.22	Illinois River, Lake Michigan
n	Illinois River, Illinois	19	3	0.2047	0.07	Upper Mississippi River
o	Duluth Bay, Minnesota	5	1	0	0	Lake Superior
	Total	249				

species limited to glaciated areas probably due to severe population bottlenecks as a result of habitat loss during glacial advances. For example, lower mtDNA diversity was observed in the lake whitefish, *Coregonus clupeaformis*, from glaciated regions in North America than in *C. lavaratus* occupying unglaciated parts of Europe (Bernatchez et al. 1989). Secondly, there is evidence in several species that mtDNA diversity is separable into a small number of distinct phylogenetic groups, which show clear geographic patterning suggestive of their origin in separate refugia. Divergent mtDNA lineages within lake whitefish (Bernatchez and Dodson 1990*a*, 1991), cisco (*Coregonus artedii*; Bernatchez and Dodson 1990*b*), walleye (*Stizostedion vitreum*; Ward et al. 1989), lake trout (*Salvelinus namaycush*; Grewe and Hebert 1988), and brown trout (*Salmo trutta*; (Bernatchez et al. 1992), have been attributed to the isolation of populations in two or more glacial refugia. The present natural ranges of these species were completely covered during each successive glaciation, except for a small northern refugium in Beringia (Lindsey 1964; Scott and Crossman 1973) and a southern refugium in the Missouri River for walleye (Scott and Crossman 1973; Cross et al. 1986).

This study extends past work by examining the impacts of glaciation on patterns of genetic diversity in a warmwater species, the brown bullhead, *Ameiurus nebulosus*, which is common throughout much of the eastern half of North America (Scott and Crossman 1973; Mandrak and Crossman 1992). *Ameiurus nebulosus* is a philopatric species (Blumer 1985) whose range now extends northward from Florida into the Great Lakes basin, westward to southern Saskatchewan, and eastward to New Brunswick. This study aimed to examine the extent and distribution of mtDNA diversity in this species with the purpose of evaluating existing hypotheses concerning its postglacial dispersal. Three putative Pleistocene refugia, in which bullheads are now common, may have contributed to recolonization of the Great Lakes region. The Atlantic Coastal Plain, east of the Appalachians, formed the Atlantic refugium (Schmidt 1986). The second region, the upper Mississippi River drainage, including the Ohio River in the northeast and the Illinois River in the northwest, formed the large Mississippian refugium (Crossman and McAllister 1986). The Missouri River drainage, including the Upper Nelson and Red rivers, was separated from the Mississippian refugium by a lobe of ice until 12 800 years ago (Cross et al. 1986) and thus formed a third distinct refugium (Bailey and Smith 1981; Crossman and McAllister 1986; Mandrak and Crossman 1992).

Conflicting hypotheses derived solely from distributional data have been advanced to explain both the glacial refugia occupied by brown bullhead and their dispersal from these refugia to colonize their current range. Underhill (1986) hypothesized that brown bullhead recolonized the Great Lakes basin solely from the Mississippian refugium, but other researchers have argued that fish originated from several refugia. Crossman and McAllister (1986) suggested that populations of this species in the northwestern Great Lakes region including the Hudson Bay drainage originated in the Mississippian refugium, whereas Mandrak and Crossman (1992) proposed a predominately Missourian origin for the same populations. Bailey and Smith (1981) and Mandrak and Crossman (1992) suggested a Mississippian drainage origin for brown bullhead now present in the central Great Lakes basin. Four studies (Bailey and Smith 1981; Hocutt et al. 1986; Schmidt 1986; Mandrak and Crossman 1992) have suggested that eastern populations of *A. nebulosus* originated from an Atlantic refugium.

Materials and methods

Two hundred and forty-nine brown bullhead were collected between 1988 and 1991 from 15 distinct sites; 12 populations from the Great Lakes drainage and 3 populations representing possible glacial refugia (Table 1, Fig. 1). Whole fish or fresh livers were transported on ice to the laboratory. Extractions of mtDNA and (or) total DNA (mtDNA and nuclear DNA) were carried out using methods from Chapman and Powers (1984) and Saghai-Maroof et al. (1984).

Restriction enzyme analysis of mtDNA

Mitochondrial DNA from each fish was digested with 12 hexameric (*Bcl*I, *Bst*EII, *Dra*I, *Eco*RV, *Hind*III, *Hpa*I, *Nco*I, *Nhe*I, *Sca*I, *Sma*I, *Ssp*I, *Stu*I) and 3 multihexameric (*Ava*I, *Hae*II, *Hinc*II) restriction endonucleases. Mitochondrial DNA fragments were electrophoretically

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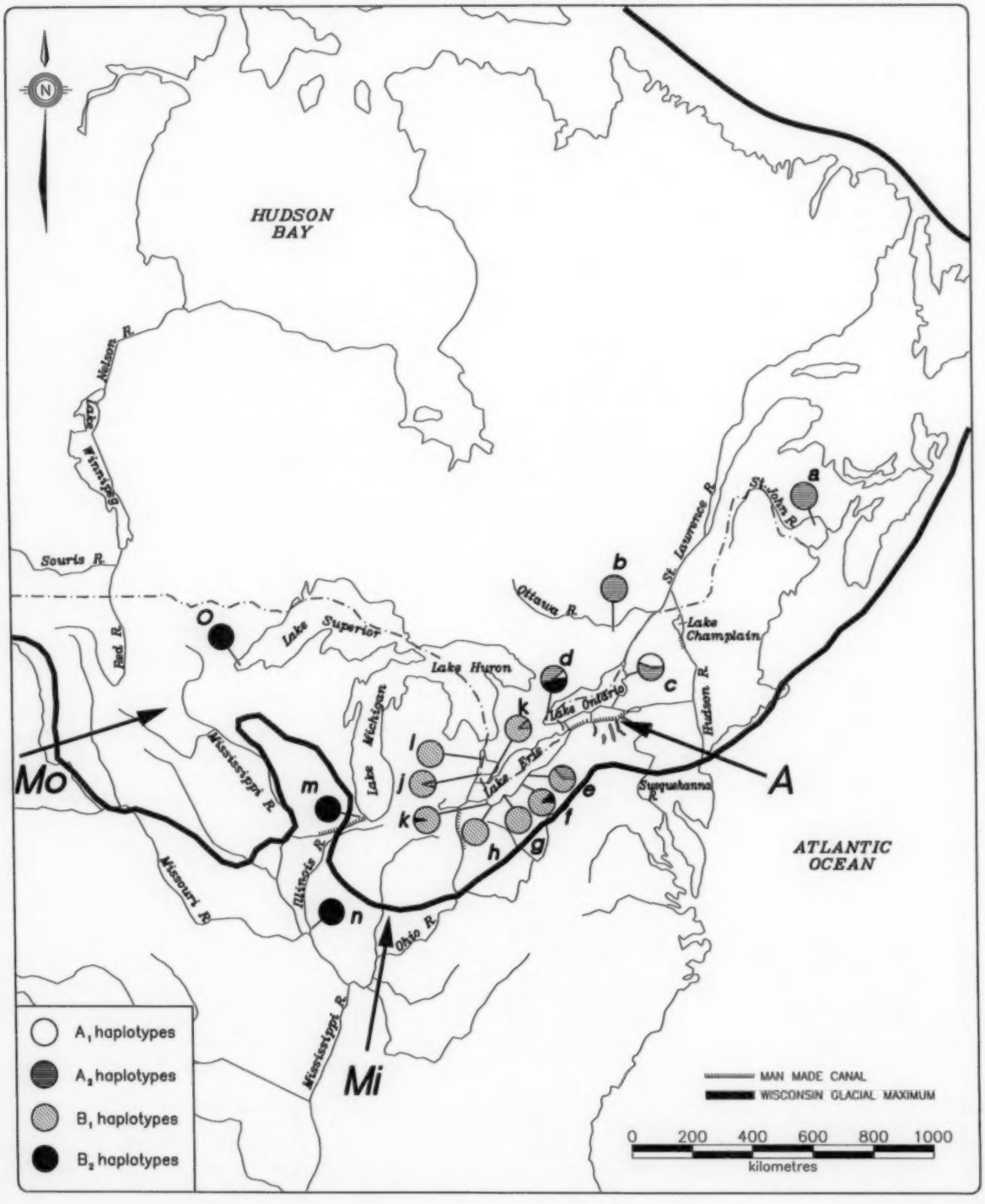
Fig. 1. Map showing locations of the 15 sample sites for brown bullhead (*Ameiurus nebulosus*) in central eastern North America. The maximum extent of the Wisconsinan glaciation within the sampling area is shown and possible general dispersal routes of brown bullhead from putative glacial refugia. A, Atlantic; Mi, Mississippian; Mo, Missourian. Arrows show direction of postglacial dispersal from refugia into the Great Lakes. Pie diagrams indicate the proportion of mitochondrial DNA haplotypes belonging to assemblages A and B and groups A_1, A_2, B_1, and B_2. See Table 1 for corresponding sample location names identified by lowercase letters. The hatched lines indicate man-made channels.



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separated in 1.2% agarose gels over 8–16 h at 25–40 V/cm and visualized under ultraviolet light using an ethidium bromide stain (Sambrooks et al. 1989). Fragments from mtDNA preparations of poor quality, as well as total DNA preparations, were transferred to nylon membrane by Southern blot (Sambrooks et al. 1989) and hybridized to a purified radiolabelled mtDNA probe. The fragments were then visualized by autoradiography. Methods of Billington and Hebert (1988) were used to prepare ultrapure mtDNA from a few samples for restriction enzyme analysis and to detect fragments smaller than 350 base pairs (bp) in length using endlabelling techniques.

Unique endonuclease fragment patterns were designated by a specific letter in order of their detection. As the analysis of brown bullhead was concurrent with a study of other ictalurid species, some letter codes for this species are not adjacent in the alphabet. Each fish was assigned a 15-letter code which described its composite mtDNA haplotype.

Data analysis

The minimum number of restriction site changes that distinguish haplotypes was estimated from analysis of fragment patterns. Fragments of identical size between patterns were assumed to have homologous restriction sites. A binary character state matrix consisting of presence and absence of restriction sites was constructed for each haplotype using the Restriction Enzyme Analysis Package (McElroy et al. 1991). The matrix was used to calculate overall percentages of shared restriction sites between pairs of haplotypes and also to estimate nucleotide differences per site (d) by the method of Nei and Tajima (1981). Based on the resulting distance matrix, a phenogram relating haplotypes by percent sequence divergence ($d \times 100$) was constructed by the Fitch–Margoliash method using the KITSCH algorithm of PHYLIP 3.3 (distributed by J. Felsenstein).

Haplotype diversity (h) was estimated according to Nei (1987), while nucleotide diversity was estimated using equations of Nei and Tajima (1981). A comparison of intrapopulation genetic diversity with interpopulation divergence was made according to Nei and Tajima (1981) and Nei (1987) using the Restriction Enzyme Analysis Package (McElroy et al. 1991). The distance matrix of net nucleotide divergence (d) among populations was used to construct a phenogram clustering all populations by the unweighted pair group method (UPGMA) (Sneath and Sokal 1973).

Finally, the combinatorial approach of Hebert et al. (1987) was used to determine the adequacy of sample sizes in this study to estimate haplotype diversity in both populations and major phylogenetic assemblages. The relationship between the number of haplotypes detected as a function of sample size was estimated by random sampling of individuals. This was carried out by an incremental choice of single fish at the population level and four fish at the assemblage level and the procedure repeated 10 times to generate an estimate of random sampling variance.

Results

Mitochondrial DNA variation

All of the 15 restriction enzymes produced polymorphic fragment patterns (Fig. 2) with a total of 142 restriction fragments generated, and a mean of 83 per individual. Fifty distinct haplotypes were revealed by these enzymes among the 15 populations (Table 2). The fragment patterns were used to estimate the number of restriction site gains or losses as required for subsequent phylogenetic analyses. Of the estimated total of 111 restriction sites, there was a mean of 80 sites per fish of which 47 were phytogenetically informative.

The size of the mtDNA genome was estimated to be 16 574 ± 207 bp (mean ± SD) by averaging the sums of all fragment patterns used for restriction site determination. This length estimate is within range of values observed in other fish species (Billington and Hebert 1991). An average of 479 bp was surveyed per fish, representing approximately 3% of the mitochondrial genome.

The relative abundance of the haplotypes was highly variable (Table 2). Haplotype 3 was most abundant, representing 16.5% of the fish sampled. This haplotype and six other haplotypes (1, 6, 22, 38, 49, 50) cumulatively made up 69% of the fish. By contrast, 32 of the other haplotypes were observed in single individuals.

Phylogenetic differentiation

The KITSCH program of PHYLIP examined over 15 000 topologies to arrive at the tree topology with the lowest percent standard deviation. The resulting phenogram contained two major phylogenetic assemblages (A and B) making up 33% and 67% of the fish, respectively, and separated by an average sequence divergence (d) of 3.22% (Fig. 3). Each assemblage was composed of two groups of haplotypes: groups A_1 and A_2 differed by an average d of 0.74%, while groups B_1 and B_2 differed by an average d of 1.07%. Two of the enzymes used in the analysis (*Ava*I, *Hinc*II) have multiple recognition sequences that overlap those of two other enzymes (*Sma*I, *Hpa*I, respectively). Their joint use does not result in a biased distance estimate, but it does give excess weight to specific character state changes or similarity. However, given the large number of sites employed in the analysis the impact on distance estimates will be small.

Geographic distribution of mtDNA phylogenetic assemblages

Strong geographic patterning was observed in the frequencies of assemblages A and B across the sampling area (Fig. 1). All fish from the three easternmost populations (a, b, c) belonged to assemblage A, whereas fish from the four sites north or west of Lake Erie (l, m, n, o) belonged to assemblage B. Assemblages A and B co-occurred in roughly equal frequency in populations d and e, while the remainder of the populations were dominated by the B assemblage.

Finer geographic structuring was observed within the assemblages (Fig. 1). A_1 haplotypes were found only at sites in Lake Ontario (c, d), while A_2 haplotypes were found at most sites except those north and west of Lake Erie. The three westernmost populations contained only haplotypes belonging to the B_2 group, while both B_1 and B_2 groups were observed in populations as far east as Lake Ontario.

mtDNA haplotype distribution and population differentiation

There was also heterogeneity in the level of haplotype diversity within populations. Several populations (a, b, f, g, n, o; Table 1) were dominated by one or two haplotypes, and this is reflected by low haplotype diversity estimates (<0.6; Table 1). In contrast, the remaining populations contained more haplotypes and had correspondingly higher haplotype diversity estimates (Table 1), approaching the maximum of 1.0 for populations c and k. Variation in levels of mtDNA relatedness within populations is reflected by the variation in nucleotide diversity estimates (Table 1). Low mtDNA diversity values were evident in populations fixed for one or two haplotypes within the same phylogenetic assemblage, while the highest

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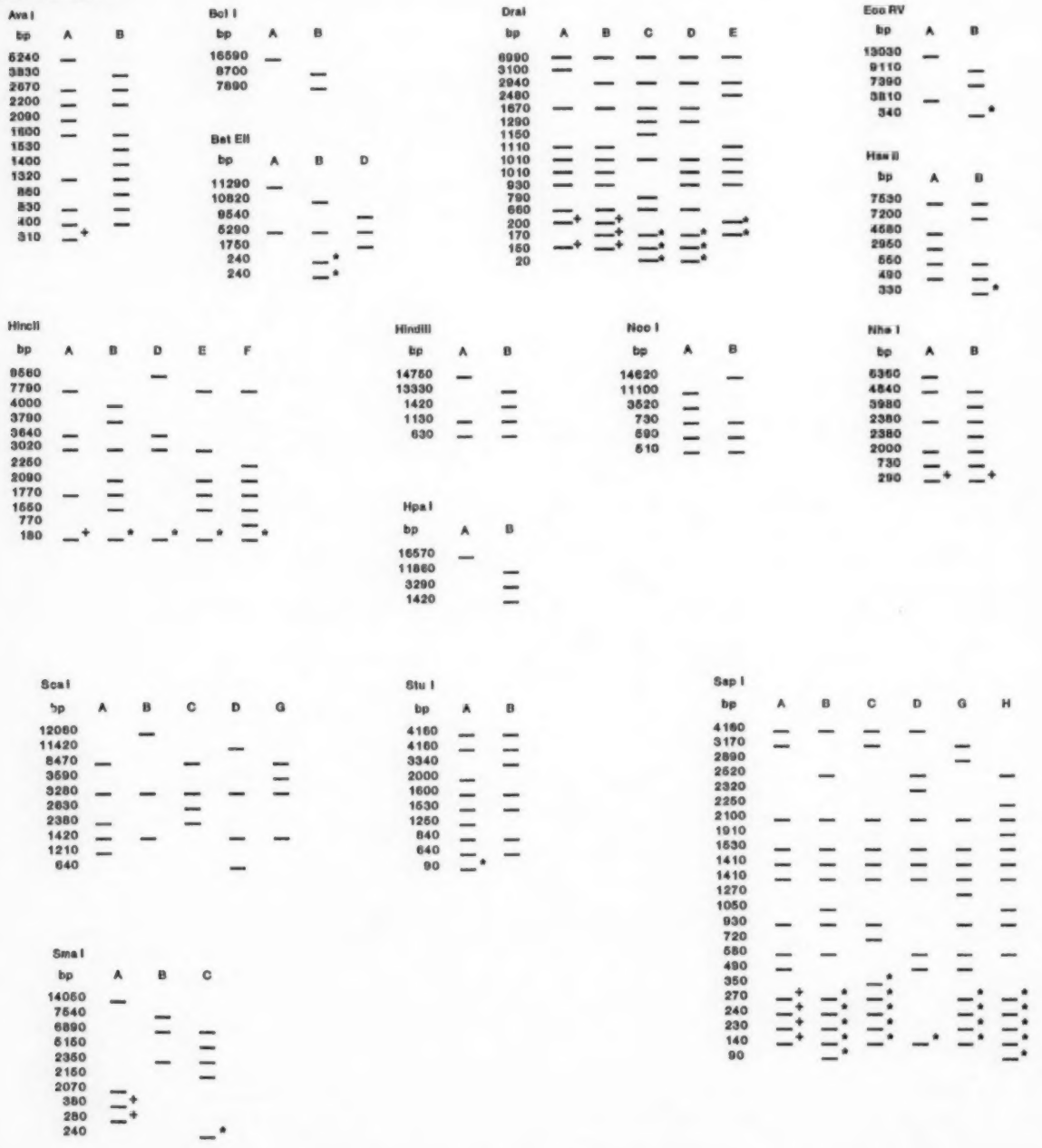
Fig. 2. Fragment size estimates of all fragment patterns observed among brown bullhead analyzed. Fragments marked with a plus sign were detected by end labelling; fragments marked with asterisks were assumed under the criterion of minimum mutational steps involved in fragment changes.



levels of mtDNA diversity were observed at sites where the two assemblages co-occurred. Four of the 32 haplotypes in assemblage B were represented in at least 3 populations (Table 2). Three of these haplotypes (1, 3, 7) were broadly distributed within the Lake Erie drainage, while haplotype 22 exhibited a disjunct distribution, being detected at both Illinois sites and at Hamilton Harbour in Lake Ontario. Within assemblage A, only haplotype 38 was widespread, being present in five populations ranging from Gibraltar Bay, Michigan, to Shogomoc Lake, New Brunswick.

Marked local differentiation of populations was observed. For example, Old Woman Creek and Huron River are less than 20 km apart, yet shared only one of 14 haplotypes. Among all populations, three haplotypes at most were shared (between

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Table 2. Composite phenotypes, frequencies (N) and distributions for each of the 50 mtDNA haplotypes detected among brown bullhead.

			No. of individuals at site														
Haplotype	Composite phenotype	*N*	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
1	AAAAAAAAAAAAAAA	27						11	1	1	8		3	3			
2	AAABAAAAAAAACA	6															
3	AAABAAAAAAAAAA	41							22		12		3	4			
4	AAADAAAAAACAAA	1											1				
5	AAABAAEAAAAAAA	1											1				
6	AABAAAEAAAAAAA	1											1				
7	AABBAAAAAAAAAA	23					7		1			12	3				
8	AAAEAAAAAAAAAA	1												1			
9	AAABABAAAAAGADA	1														1	
10	AAADAAAAAAAAAA	2									1			1			
11	AAAEAADAAAAAAA	1									1						
12	AABBAAAAAAAGADA	1														1	
13	AAABABAAAAAAADA	1									1						
14	AAAAAAABAAAAAAA	1									1						
15	AAAAAAAAAAAGAAA	1							1								
16	AABAAAAAAAAAACA	9								1		8					
17	AABBAADAAAAAACA	1								1							
18	AABEAAAAAAAAACA	1								1							
19	AAABAADAAAAAACA	1								1							
20	AAAEAAAAAAAAACA	1								1							
21	AAABAAAAAAACACA	1								1							
22	AADBABAAAAAGADA	25				7									1	17	
23	AAABAAAAAAAGADA	1				1											
24	AADBAAABAAAGADA	1				1											
25	AABBAAAAAAAAABA	1					1										
26	AABBAAAAAAAAAGA	1										1					
27	AABBAAAAAAACAAA	1										1					
28	AABBABAAAAAGADA	2													2		
29	AABBABAAAAAAADA	1													1		
30	AAABAAAAAAABAAA	2					2										
31	AAABAAAAAAABACA	3					3										
32	AADBAAAAAAAGADA	5															5
33	BBBCBBABBBBBBBB	1											1				
34	BBBCBBBBBBBDBBB	1											1				
35	BBBCBBBABBABBBB	1									1						
36	BBBCBBEBBBBABHB	1		1													
37	BBACBBEBBBBBBBB	6			1	3	2										
38	BBBCBBEBBBBBBBB	24	11		1	6	5					1					
39	BBBCBBBBBBBBBB	2					1					1					
40	BBABBBEABAAGBBB	1			1												
41	BBABBBEBBBBBBBB	4			2	2											
42	BAABBBEBBBBBBBB	1			1												
43	BBABBBEBBBBBBBB	2			2												
44	BAACBBFBBBBBBBB	1			1												
45	BAACBBEBBBBBBBB	1			1												
46	BAACBBEBBBBBCBB	1			1												
47	BBACBBEBBBBBABB	1			1												
48	BBACBBEBBBBBBBA	1			1												
49	BBBCACEBBBBBBBB	15	15														
50	BBBCBBEBBBBABBB	19		19													

Note: Haplotypes 1–32 belong to mtDNA assemblage B, while haplotypes 33–50 belong to assemblage A. Composite phenotypes refer to fragment patterns described in Fig. 3 for the following 15 enzymes: *Ava*I, *Bcl*I, *Bst*EII, *Dra*I, *Eco*RV, *Hae*II, *Hin*cII, *Hin*dIII, *Hpa*I, *Nco*I, *Nhe*I, *Sca*I, *Sma*I, *Ssp*I, and *Stu*I. Distribution codes refer to sample locations in Table 1 and Fig. 3.

populations i and l, e and j, c and d), while two populations shared no haplotypes with other sites (b, o).

Although few haplotypes were shared between neighbouring populations, there was little nucleotide differentiation among haplotypes within a local geographic region. Figure 4 illustrates the genetic relationships among brown bullhead

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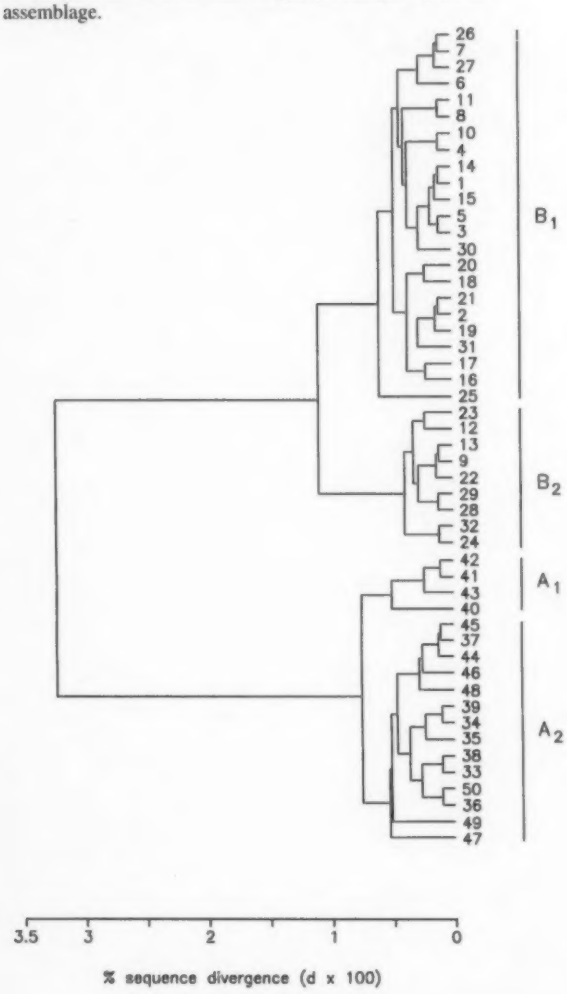
Fig. 3. Genetic distance phenogram generated by KITSCH (PHYLIP 3.3), showing percent sequence divergence per site among brown bullhead mitochondrial DNA haplotypes based on shared restriction sites. The numbers 1–50 refer to haplotypes defined in Table 2. Capital letters identify assemblages while subscripted numerals indicate the groups comprising each assemblage.

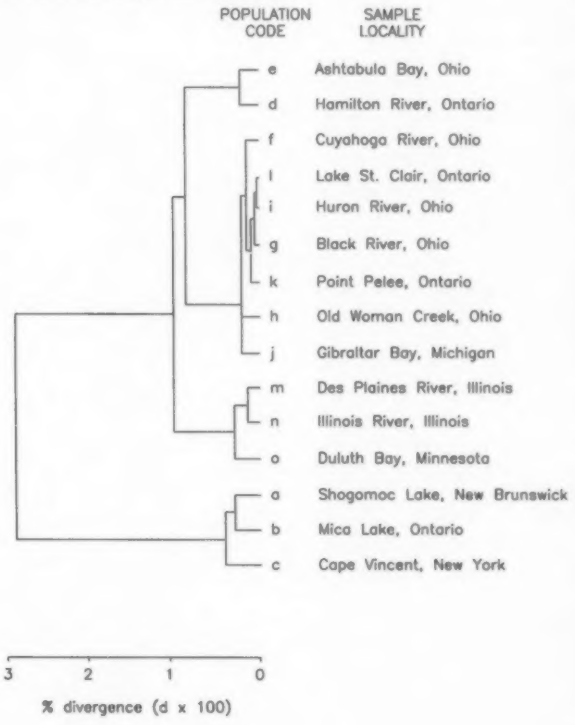


populations. Populations in the Lake Erie basin are predominately composed of closely related B haplotypes and so group together (d = 0.21%; populations f, l, i, g, k, h, j). The sites where the two assemblages co-occur formed a distinct cluster (d = 0.88%; populations e, d) and were more closely related to the Lake Erie basin populations than to the cluster of three westernmost populations (d = 1.01%; populations m, n, o), which contained only haplotypes in assemblage B_2. The eastern populations grouped together (d = 0.42%; populations a, b, c) as a distinct cluster from all others (d = 2.91%), reflecting their domination by haplotypes in assemblage A.

Effectiveness of individual surveys in detecting mtDNA variation

There was no clear difference in overall mtDNA haplotype diversity between phylogenetic assemblages A and B. Figure 5 illustrates the relationship between the number of mtDNA haplotypes in each assemblage as a function of sample size. Using Nei's (1987) haplotype diversity index, h values within assemblages A and B were estimated to be 0.83 and 0.86, respectively, out of a possible 1.0. Thus, the number of haplotypes found for a given sample size was high and approximately the same for both assemblages.

Fig. 4. UPGMA phenogram clustering brown bullhead populations by comparing intrapopulational genetic diversity to interpopulational divergence. Lowercase letters refer to sample locations (see Fig. 1 and Table 1).



For intrapopulation analysis of haplotype diversity the populations were divided into three groups based on their constituent assemblages (Fig. 6). In the single assemblage populations there was no consistent association between sample size and number of haplotypes detected. However, for the mixed assemblage populations, an increase in the number of haplotypes was detected with increasing sample size, excepting two populations (d, e) that reached an asymptote.

Discussion

The present study has revealed considerable mtDNA divergence and variation among brown bullheads in the Great Lakes basin. The level of sampling in this study was sufficient to reveal a high level of divergence within the species, relative to other fish species, as well as considerable population differentiation. Two distinct haplotype assemblages were resolved, which were themselves each divided into two groups. Component haplotypes of assemblages A and B differ in nucleotide

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Fig. 5. Effect of sample size on the number of mitochondrial DNA haplotypes detected in the two major phylogenetic assemblages identified in Fig. 1.

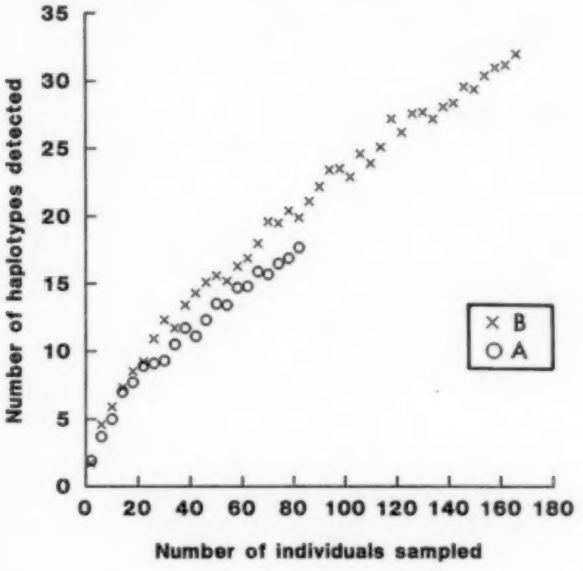
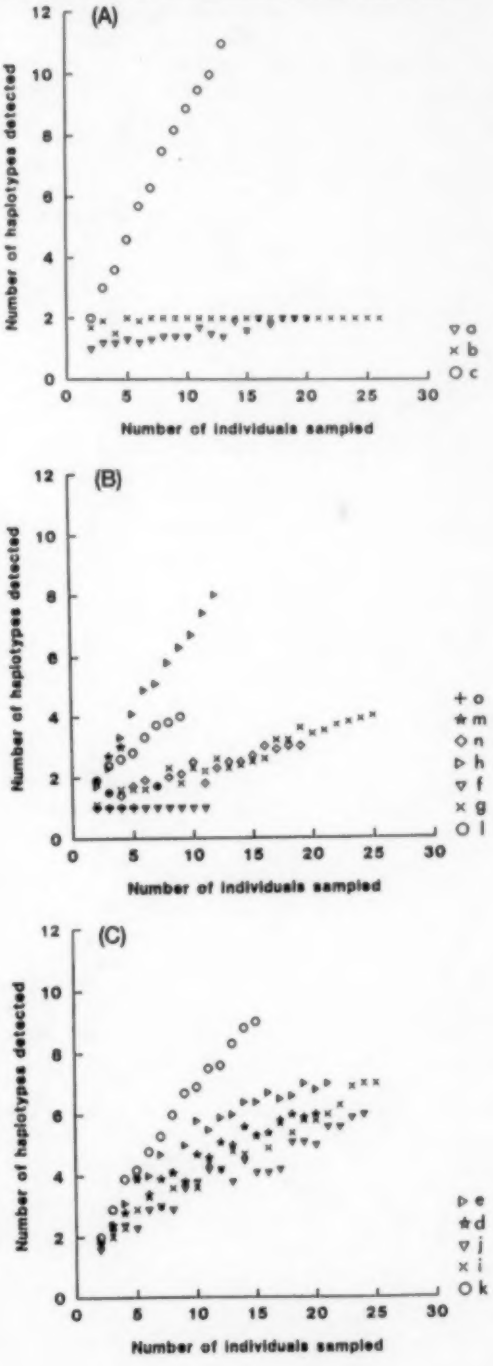


Fig. 6. Effect of sample size on the number of mitochondrial DNA haplotypes detected within populations. Populations were grouped according to the phylogenetic assemblages they contained.



site divergence by an average of 3.22%, a level of divergence greater than that detected among northern freshwater fishes (Billington and Hebert 1991). However, similar and higher divergence values have been reported within bowfin (*Amia calva*) and several sunfish species (*Lepomis* spp.) in the southern United States (Avise et al. 1984; Bermingham and Avise 1986).

If mtDNA in brown bullhead evolves at the same rate as that estimated for primates and birds (Brown et al. 1979; Shields and Wilson 1987), then the two major assemblages last shared a common maternal ancestor approximately 1.6 million years ago, a divergence time that coincides with the Pliocene–Pleistocene boundary. Using this calibration, the two groups of assemblage A originated some 400 000 years ago, while the groups composing assemblage B diverged 600 000 years ago during the Pleistocene. In comparison, using the same calibration, the estimated divergence times for major haplotype lineages in lake trout are 270 000 – 500 000 years ago (Grewe and Hebert 1988); in walleye, 230 000 years ago (Billington and Hebert 1988); and in lake whitefish, 150 000 years ago (Bernatchez and Dodson 1991). Although there is evidence for rate heterogeneity in mtDNA divergence among taxa, which may challenge use of the primate–bird calibration (Martin et al. 1992), it is likely that the relative differences apparent in lineage age among species are valid. This result suggests that surviving lineages of most northern fish species were confined to a single refugium in the mid-Pleistocene but persisted in several refugia in the late Pleistocene, while warmwater species such as brown bullhead retain mtDNA variation that originated in the Pliocene, as well as lineages that diverged in the mid-Pleistocene.

The high level of haplotype diversity within brown bullhead (0.93 out of a maximum of 1.0) may have been fostered by the low vagility of this species (Blumer 1985), which enhances population subdivision and may act as a buffer against lineage extinction (Avise et al. 1988). This characteristic probably also slowed the diffusion of fish from single

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refugia, thus setting the stage for a high degree of geographic patterning of lineages. The current geographical distributions of the mtDNA groups are consistent with their allopatric origin in separate Atlantic, Mississippian, and possibly Missourian refugia. Hence, the two groups (A_1, A_2) in assemblage A have predominately eastern distributions, which suggests their origins in the unglaciated regions east of the Appalachians on the Atlantic coast. The presence of two groups of haplotypes within this assemblage could result from the divergence of lineages from a common ancestor followed by extinction of intermediate lineages (Avise et al. 1987). This hypothesis would not, however, explain the apparently nonrandom geographic distribution of A_1 and A_2 haplotypes, with the A_1 haplotypes found only in Lake Ontario. A more likely scenario to explain their geographic patterning is regional isolation within the Atlantic refugium. Schmidt (1986) proposed the existence of a Pleistocene refugium on now submerged banks off the coasts of the New England States and the Atlantic Provinces. Bernatchez and Dodson (1991) suggested that this region was the source of the distinct "Acadian" race of lake whitefish. However, there is now substantial evidence (Piper et al. 1990; G. Fader, Atlantic Geoscience Centre, Bedford, N.S., personal communication) that Wisconsinan ice sheets extended across these banks until approximately 16 000 years ago. In particular, the Georges Bank, south of the Gulf of Maine, supported a boreal ecosystem with cool-water habitats for only a brief period until rising seas submerged it about 8500 years ago (G. Fader, personal communication). Furthermore, as the Gulf of Maine was much larger than at present while the Georges Bank was above water (Piper et al. 1990) it precluded the possibility of northward migration by species with low salinity tolerance such as the brown bullhead. Therefore, brown bullhead in the Atlantic region may have occupied a fragmented refugium on the Atlantic Coastal Plain in the boreal Pleistocene habitat that existed on the emergent continental shelf off south Virginia and North Carolina (Hocutt et al. 1986).

Two major routes were available for northward dispersal from this region. The first route involved rivers in the Delaware–Hudson drainage that had temporary post-Wisconsinan connections (Hocutt et al. 1986). The Hudson River provided access from the Atlantic Coastal Plain to the eastern Great Lakes basin and also farther north via Lake Champlain to the St. Lawrence basin (Bailey and Smith 1981; Schmidt 1986; Underhill 1986; Mandrak and Crossman 1992). The Susquehanna River, which originated in the Chesapeake drainage, briefly provided a second pathway to Lake Ontario (Bailey and Smith 1981; Underhill 1986) from regions south of the Delaware–Hudson drainage, and perhaps south of the Coastal Plain itself (Hocutt et al. 1986).

The broad distribution of A_2 haplotypes within both the eastern Great Lakes and their presence in fish from New Brunswick suggests that they had more than one dispersal pathway from the Atlantic, perhaps through the upper west and upper north branches of the Hudson River. Access to the eastern states and provinces may have been through extensive interconnection of streams that drained away the glacial meltwaters along the eastern seaboard (Schmidt 1986). The more limited distribution of A_1 haplotypes suggests that fish with this lineage had fewer access routes to the Great Lakes region, perhaps via the brief Susquehanna connection approximately 13 000 years ago (Bailey and Smith 1981). To evaluate these hypotheses, more detailed studies are required of mtDNA diversity in brown bullhead from the southeastern United States.

Regional isolation was probably also the driving force behind the origin of the two groups (B_1, B_2) that constitute assemblage B. Physical barriers may have fragmented the Mississippi refugium, or alternatively, fish may have persisted in both the Missouri and Mississippi drainages. B_1 haplotypes were detected in the Lake Erie drainage and Lake St. Clair, while B_2 haplotypes had a predominately western distribution but were found throughout the Great Lakes basin. The distributional patterns suggest that the diverse B_1 assemblage arose in the Mississippian refugium and colonized the Great Lakes via the early Ohio River connection to glacial lakes in the Huron–Erie basin, approximately 14 000 years ago. Stewart and Lindsey (1983) have argued that ictalurids were unlikely to survive in proglacial lakes, implying their more recent dispersal into the Great Lakes basin, but fossil material confirms their presence in the proglacial lakes 12 500 years ago (Mandrak and Crossman 1992). Mandrak and Crossman (1992) also noted, however, that brown bullhead were probably confined to the southern parts of glacial lakes away from the ice margin. The absence of B_1 lineages in the western populations suggests that ancestral fish containing this lineage did not make use of the dispersal route between the Michigan basin and the Illinois–Mississippi drainage or ancient connections across the Michigan peninsula (Underhill 1986).

The origin of the divergent B_2 assemblage is puzzling because of its disjunct distribution. This assemblage was fixed in the three westernmost populations, rare in Lake Erie, but present at a frequency of 0.4 in Hamilton Harbour (Lake Ontario). Its dominance at western sites suggests its origin from either a Missourian or a northwestern Mississippi River refugium, separate from that occupied by the B_1 group. During the Wisconsinan glaciation there were no obvious barriers to dispersal within the unglaciated Mississippi drainage, but considerable evidence exists for a distinct Missourian refugium. Prior to the Pleistocene the Missouri River drained northward into Hudson Bay (Crossman and McAllister 1986), and during the Pleistocene the upper Missouri River was separated from the northwestern branch of the Mississippi River by an ice lobe (Cross et al. 1986). As the Sheyenne and Red River outlets to glacial Lake Agassiz in the north (Bailey and Smith 1981; Mandrak and Crossman 1992), and a direct connection was made 12 800 years ago, south of the confluence of the Illinois and Mississippi rivers, which remains intact. Therefore, the most probable origin for the divergent B_2 assemblage is the Missourian refugium.

Populations in the Missouri drainage had extensive opportunities to enter the Great Lakes basin. The presence of brown bullhead in rivers of the Hudson Bay drainage once covered by Lake Agassiz (Mandrak and Crossman 1992) suggests that they could have made use of northern outlets to the Superior basin and subsequently dispersed within glacial lakes connecting the Michigan, Huron, and Erie basins. From these lakes, brown bullhead of the B_2 group may have gained access to Lake Ontario via the Kirkfield outlet across the present day Kawartha Lakes area (Bailey and Smith 1981). This conflicts with the proposal (Crossman and McAllister 1986) that brown bullhead in the Hudson Bay drainage originated from the

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Mississippian refugium. Alternatively, brown bullhead in the lower Missouri River could have reached Lake Michigan through the intermittent but long-term Illinois–Mississippi connection. This route seems more likely given the current rarity of brown bullhead in the northwestern drainages of the Missouri River and their prevalence in the Arkansas River drainage of the western Mississippi basin (Scott and Crossman 1973). However, their presence in the latter drainage is questionable (Cross et al. 1986) because of taxonomic confusion with the black bullhead (*Ameiurus melas*), which is ubiquitous in the western Mississippi basin. There is therefore some uncertainty as to a dispersal source of brown bullhead from within the Missourian refugium, but this could be resolved by the careful analysis of individuals from the Arkansas drainage.

The limited zone of co-occurrence of the two major assemblages suggests that the brown bullhead did not make extensive use of ancient connections that joined the Ontario basin with the eastern end of glacial Lake Algonquin in the north and with Lake Erie to the east through the Niagara River. The limited assemblage overlap is probably not due to poor dispersal capabilities of the species because evidence from this study suggests that brown bullhead are capable of dispersing over long distances in recent evolutionary time. For example, haplotypes 22 and 38 were each detected in populations more than 800 linear kilometres apart. Alternatively, ecological characteristics of brown bullhead might help to explain the limited zone of overlap. As Mandrak and Crossman (1992) and Stewart and Lindsey (1983) suggested, temperature preferenda may have limited the brown bullhead to the southern margins of glacial lakes, away from the northern connecting channels. The Niagara River was an effective recolonization route for fish populations for only a short time before the Niagara Falls became a barrier to dispersal approximately 10 000 years ago (Underhill 1986). Interestingly, limited sampling of brown bullhead from Lake Sasajewun (M. Murdoch, unpublished data), in an area covered by outlets to the Ottawa River of glacial Lake Algonquin and later Nippissing, indicated mtDNA restriction fragment patterns that suggest their origin from fish that dispersed from the Atlantic refugium. However, eastern brown bullhead apparently did not gain access to the western Great Lakes through this route as no Atlantic assemblage haplotypes were detected north or west of Gibraltar Bay in the Detroit River. Additional evidence to support this was provided by a limited sampling of brown bullhead from Saginaw Bay in Lake Huron, which revealed restriction fragment profiles indicative of B assemblage haplotypes (M. Murdoch, unpublished data).

The restricted region of assemblage overlap may reflect very recent dispersal. Underhill (1986) suggested that man-made diversions in the Great Lakes may have played a significant role in the distribution of some species. The New York – Erie Barge Canal re-opened ancient westward connections from the Hudson River to Lake Ontario and provided a route to the Niagara River at the eastern end of Lake Erie. The Welland Canal, which bypasses Niagara Falls, opened a dispersal route between Lake Ontario and Lake Erie. These diversions may explain the overlap of assemblages A and B at either end of the Niagara River. The Atlantic assemblage was found at a frequency of 0.38 in Ashtabula Harbour but was rare at sites farther west, while the possible Missourian assemblage was detected at Hamilton Harbour at a frequency of 0.4 but not found farther east. Furthermore, haplotypes 37 and 38 were found in approximately equal frequency at both sites. The co-occurrence of only group A_2 with the B assemblage supports the hypothesis of the Hudson River being the primary route for dispersal group A_2 haplotypes into the eastern Great Lakes.

In summary, brown bullhead demonstrate the classic phylogeographic pattern in which members of geographically separated populations occupy different branches of an intraspecific phylogenetic tree (Avise et al. 1987). Assemblage A dominated populations east of the Niagara River, while populations west of the Niagara River were dominated by assemblage B. The similarity in phylogeographic patterns observed among lake whitefish, lake trout, walleye, and brown bullhead in the Great Lakes region strongly suggests that the Pleistocene glaciations played a common role in the geographic distributions of their mtDNA lineages. In contrast to these highly mobile, cold-tolerant species, the Pleistocene glaciations did not greatly restrict the range of brown bullhead, and as a result, much of its historical mtDNA variation and divergence was preserved in populations living south of the ice sheets.

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Abundance and condition of larval cod (*Gadus morhua*) at a convergent front on Western Bank, Scotian Shelf

Steve E. Lochmann, Christopher T. Taggart, David A. Griffin, Keith R. Thompson, and Gary L. Maillet

Abstract: In November and December 1992, Atlantic cod (*Gadus morhua*) larvae were most abundant at a convergent front located at the periphery of a well-mixed gyrelike water mass rotating near the crest of Western Bank (outer Scotian Shelf). Zooplankton wet biomass and plankton abundance (272 and 529 μm size-classes) were also higher in the frontal region relative to the adjacent water masses. We used the frontal feature to test the hypothesis that larvae in frontal regions are in better condition than larvae elsewhere. No significant differences in triacylglycerol content (an index of nutritional condition), Fulton's *K* condition index, nor in the daylight feeding ratio were found between larvae in the frontal region and those in the adjacent waters. The convergent front acted as a larval collector, but exchange with other water masses eliminated measurable differences in larval condition. Our observations indicate that physically driven retention, not differential mortality (approximated by condition), was responsible for high abundances of cod larvae at this front.

Résumé : En novembre et décembre 1992, les larves de morue (*Gadus morhua*) étaient les plus abondantes dans un front convergent situé à la périphérie d'une masse d'eau bien brassée en rotation, semblable à un tourbillon, près de la crête du banc Western (plate-forme Scotian extérieure). La biomasse humide de zooplancton et l'abondance du plancton (classes de taille de 272 et 529 μm) étaient également plus élevées dans la région frontale par rapport aux masses d'eau adjacentes. Nous avons utilisé la caractéristique frontale pour vérifier l'hypothèse que les larves dans les régions frontales sont en meilleur état physique que les larves vivant ailleurs. Aucune différence statistiquement significative dans la teneur en triacylglycérol (un indice de l'état nutritionnel) dans l'indice *K* de l'état physique de Fulton ni dans le rapport d'alimentation à la lumière naturelle n'a été observée entre les larves vivant dans la région frontale et celles qui vivent dans les eaux adjacentes. Le front convergent a agi comme collecteur de larves, mais l'échange avec d'autres masses d'eau a éliminé les différences mesurables touchant l'état des larves. Nos observations ont indiqué que la rétention à motivation physique, et non la mortalité différentielle (telle qu'établie de manière approximative par l'état), était responsable de l'abondance élevée de morues dans ce front.
[Traduit par la Rédaction]

Introduction

Strong positive correlations between larval fish abundance and the abundance of their prey are frequently observed at hydrographic fronts (e.g., Richardson et al. 1986; Govoni et al. 1989; Sabates and Maso 1990). One explanation for high abundances of ichthyo- and zoo-plankton at fronts is higher survival due to better growth in enhanced feeding environments (Taggart et al. 1989; Brandt 1993). Buckley and Lough (1987) noted that prey levels were higher and haddock larvae exhibited better growth in stratified water masses near the shelf slope front than in well-mixed water masses inside the 60-m isobath on Georges Bank. An alternative explanation for the higher levels of plankton at fronts is that passive transport and convergence accumulate larvae and their prey in frontal regions (Govoni et al. 1989).

Several hypotheses attempt to explain recruitment variability through interactions between the early life history of marine fish and their local feeding environment (Hjort 1914; Cushing 1972; Lasker 1975). These hypotheses predict that strong year-classes may result from positive temporal or spatial correlations between larval fish and their prey. However, correlations between the abundance of fish larvae and their food could be associated with large year-classes through several mechanisms. Enhanced feeding in a high food environment may lead to improved nutritional condition and increased survival. Alternatively, physical processes responsible for the retention of zoo- and ichthyo-plankton in a particular geographic region could lead to strong year-classes in, or from, that region regardless of whether condition and survival are enhanced through increased predator–prey interactions. However, examining these predator–prey interactions in the field, where

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physical process can have an enormous influence, has scale-related problems that require careful consideration (Taggart and Frank 1990).

Hydrographic fronts and their surrounding waters masses can be viewed as "natural laboratories" where the influence of enhanced prey abundance on larval abundance, condition, and (or) survival can be studied at small time and space scales that match the scale of the governing physical and biological processes. However, to draw conclusions about growth, survival, and recruitment in relation to frontal features and to provide for improvements in our predictive capabilities, it is necessary to determine not only the reasons for high abundances but also what benefits, if any, larvae derive from frontal residence. Of course the nutritional benefit of occupying an enhanced food environment such as an oceanographic front may be reduced if the front is ephemeral or larval residence in the frontal region is brief. Thus, fronts offer the opportunity to quantify the relative importance of physical and biological processes leading to observations of high larval abundance and to quantify the links between the feeding environment, nutritional condition, and survival under naturally occurring conditions.

The Ocean Production Enhancement Network (OPEN) research programme was designed, in part, to quantify directly in the field the variability in several characteristics of cod larvae in time and space and the physical processes that are thought to directly influence that variability (Anonymous 1995). During one of OPEN's ichthyoplankton surveys on the eastern Scotian Shelf we observed a maximum in the abundance of Atlantic cod (*Gadus morhua*) larvae in a frontal region near the crest of Western Bank and took the unique opportunity to ask whether cod larvae in the frontal region were in better condition than those larvae residing in the surrounding water masses. This analysis is part of a larger collaborative study designed to determine the characteristics of cod larvae that survive the larval period by tracking a water mass and continuously re-sampling a group of larvae resident therein.

In this paper our assessments of feeding and nutritional condition are based on gut content, gravimetric or morphometric, and lipid biochemistry measures.We present the relevant hydrographic data describing the water mass structure near the crest of Western Bank and describe its relationship to various biological measures. Finally, we provide a conceptual model of the frontal processes that is consistent with the biological and physical observations.

Materials and methods

Field

Physical and biological data were collected during the Petrel V Cruise No. 92-31 on Western Bank (Fig. 1*a*) during the period 22 November – 16 December 1992 (Griffin and Lochmann 1993). A complete description of the technical aspects of the cruise are presented elsewhere (Bowen et al. 1995; Taggart et al. 1996; Griffin and Thompson 1996). Here we provide a description of the aspects that are relevant to this frontal study, and we do so in a generally chronological order so that the reader can appreciate the real-time evolution of the study.

We began with an initial mesoscale survey comprised of three sampling transects on Western Bank during 23–25 November (Fig. 1*b*) to map the temperature, salinity, and density fields and the abundance distribution of larval cod. On each transect temperature and salinity profiles were collected at ~7 km intervals with a Seabird™-25 conductivity–temperature–depth sensor (CTD), and plankton samples were collected at ~7- to ~21-km intervals with a 60 cm diameter bongo net sampler (Posgay and Marak 1980) fitted with 333 μm mesh nets. Bongo nets were towed at a nominal ship speed of 1 $m \cdot s^{-1}$ in a continuous double oblique manner (~1 $m \cdot s^{-1}$ wire speed) to depths of between 5 and 10 m above the bottom. Depth was determined by the length of tow wire deployed and its incident angle. The average volume filtered per net, for each deployment, was 150 m^3 and was monitored using General Oceanics™ flow meters. Cod larvae from plankton samples collected during the initial survey were immediately sorted after net recovery, enumerated, and preserved in 95% ethanol.

The initial survey revealed several water masses in the region (see Results below and Taggart et al. 1996; Griffin and Thompson 1996), one of which was termed crest water (CW) located near the crest of the bank. This water mass was well mixed and of intermediate temperature and salinity relative to the surrounding water masses, and because it contained high abundances of larval cod, it was targeted for water mass tracking.

Tracking was achieved by using the Ocean Probe real-time oceanographic monitoring system (see Bowen et al. 1995 for complete details) which included shipboard and moored telemetering instrumentation as well as telemetering Loran-C drifters (Sanderson 1995). The Ocean Probe system delivered oceanographic data in real-time to a data-assimilative hydrodynamic model (Griffin and Thompson 1996) which produced time-dependent flow fields within a 60 × 60 km model domain of the study area. By employing this model (updated at least twice daily) in a Lagrangian frame of reference the flow fields could be used to assess the present and predict the future positions of previous sampling locations as they were advected around and across the bank within the moving water mass. Thus, we were able to track the aggregation of larval cod found within the crest water.

One radio-telemetering Loran-C surface drifter with a 2 × 10 m window-blind panel drogued at a depth of 20 m was deployed near the crest of the Bank on 25 November where the local larval cod abundance maximum was found. Thirteen similarly configured drifters were subsequently deployed in an evenly spaced pattern around the central drifter within the ~20 km diameter CW containing the larval cod (Sanderson 1995).

Subsequent to the initial survey 25 oblique bongo samples and 27 vertical profiles of zooplankton were collected during the 25 November – 3 December period. Vertical profiles were conducted using a 1-m^2 EZNET™ (Open Seas Instruments, Musquodoboit Harbour, N.S.), the marketed version of the Bedford Institute of Oceanography Net and Environmental Sampling System (BIONESS; Sameoto et al. 1980). The EZNET was fitted with ten 333-μm mesh nets, General Oceanics digital flow meters (internal and external), pitch and roll sensors, a Seabird-19 CTD, and an Optical Plankton Counter™ (OPC; Focal Technologies Inc., Dartmouth, N.S.). The EZNET was used to sample discrete depths in a stepped oblique manner at 5- or 10-m intervals (depending on bottom depth) to within 10 m of the bottom. Towing speed averaged 1 $m \cdot s^{-1}$ and each net was deployed for ~5 min and the filtered volume averaged 250 m^3 per net. Plankton samples other than those collected during the initial bongo survey were processed as follows. After recovery the nets were rinsed to the codends and samples were immediately sorted for cod larvae. Ten larvae (when available) from a single bongo net, or from each EZNET net, were videotaped through a dissecting microscope for morphometric analysis (Miller et al. 1995) and individually preserved in liquid nitrogen for biochemical analyses. The remaining larvae were preserved in ethanol in bulk, and the plankton samples were preserved in 4% $MgCO_3$ buffered formalin in seawater.

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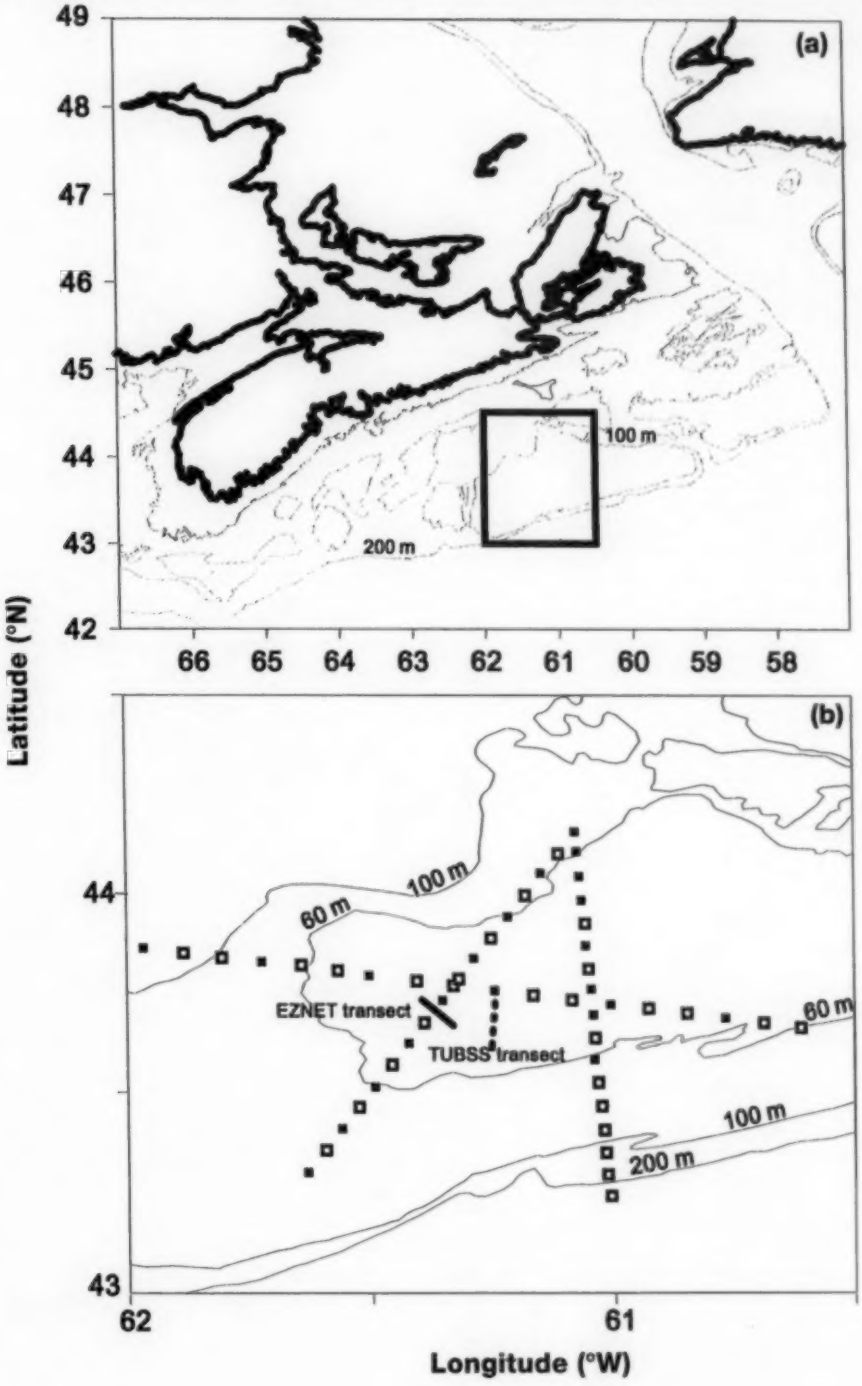
Fig. 1. (*a*) Bathymetric chart of the Scotian Shelf showing the 100- and 200-m isobaths with the Western Bank sampling area outlined. (*b*) Enlargement of sampling area showing the 60-, 100-, and 200-m isobaths, the locations of CTD (open symbols) and CTD–bongo (solid symbols) stations from the initial survey, the EZNET frontal transect (solid line), and the TUBSS frontal transect (broken line).



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A severe storm interrupted the study from 3 to 6 December. Although tracking and sampling continued between 7 and 16 December, we focus here on data collected between 23 November and 3 December when the frontal feature was studied.

A fine-scale sampling transect, across and normal to the front, was conducted on 29 November using the EZNET (Fig. 1*b*). Sampling was accomplished by sequentially towing each of the 10 EZNET nets at a fixed depth of 12.5 m, for 5 min, over a distance of ~330 m, at a towing speed of 1 $m \cdot s^{-1}$, beginning in the tracked CW. The fixed sampling depth was chosen because it was the depth of maximum larval cod abundance according to our previous sampling efforts (see also Fig. 8). We subsequently conducted a 12 km frontal transect on 29 November using the undulating Towed Underwater Biological Sampling System (TUBSS). TUBSS included an OPC, an Ocean Sensors™ CTD, and a Variosens™ fluorometer (see Sprules et al. 1992). The TUBSS frontal transect (Fig. 1*b*) was also normal to the front and was located approximately 10 km east of the location of the EZNET frontal transect.

Laboratory

Abundance and biomass estimates

Plankton collections were examined again in the laboratory and any remaining cod larvae were removed, enumerated, and preserved in 95% ethanol. The remainder of each sample was then washed and concentrated on a 200 μm mesh preweighed sieve, towel-dried to remove excess water, and weighed to the nearest gram to obtain wet biomass estimates. In all EZNET deployments, except the fine-scale frontal transect, the towing profile was stepped oblique. For a direct comparison between the depth-integrated bongo samples and the discrete-depth EZNET samples, zooplankton wet biomass and larval abundance estimates from the latter were depth averaged, though weighted by the volume filtered. Larval abundance was standardized to larvae per 100 m^{-3} and zooplankton wet biomass was standardized to grams per cubic metre.

Gut content and feeding ratio

Cod larvae initially preserved in liquid nitrogen were thawed and their stomachs removed for gut content analysis. This procedure also avoided contamination of stored lipid measures in larvae by lipids found in the undigested prey in the gut (Lochmann et al. 1996). Micro-zooplankton in the gut were enumerated and identified to the lowest possible taxonomic level (McLaren and Avendano 1995). As the functional response of cod larvae to their prey field varies with prey abundance, an index of gut fullness is not a sufficiently direct measure of feeding rate (Munk 1995). Nonetheless, we did calculate the feeding ratio (average number of prey per larva) among sample groups. This was done not to directly assess differences in feeding rates among samples but to use as an index of recent feeding activity (Powell et al. 1990). The index can also provide evidence of a link between the prey field and larval nutritional condition (Canino et al. 1991).

Biochemical condition

To quantify lipid-class composition and the defatted dry weight (DDW) of the thawed larvae, each was placed into 1 mL of dichloromethane–methanol solvent (2:1 v/v, HPLC grade) and extracted for approximately 24 h. Five micrograms of nonadecane (internal standard) were added to each extraction tube to estimate recovery efficiency. Samples were kept on ice prior to evaporation under purified nitrogen gas and reconstituted twice in 10 μL of extraction solvent. The remaining larval carcass was dried to a uniform weight and weighed to the nearest 0.1 μg using a Cahn Gram Electrobalance (Model G, Ventron Corp., Paramount, Calif).

Lipid class measurements were made using TLC–FID (thin-layer chromatography – flame ionization detection) with a Chromarod-Iatroscan™ Mark V (Iatron Laboratories Inc., Tokyo, Japan; Ackman et al. 1990). We used a RSS/ANCAL A/D card and T DATASCAN software(version 2; RRS, Inc., 3176 Pullman Street, Suite 110, Costa Mesa, CA 92626, U.S.A.) for data acquisition and analyses.

We examined quantities of sterol esters, triacylglycerol (TAG), free fatty acids, diacylglycerol, sterols, and phospholipids in each cod larva. For each larva, 5–10 μL of extract was spotted on an S-III Chromarod (Iatron Laboratories Inc., Tokyo, Japan) using a microsyringe. Chromarods were first developed in hexane – diethyl ether – formic acid (67.9:2.1:0.04, v/v/v) for 33 min and then dried for 2 min at 60°C and partially scanned to detect the internal standard and sterol esters. Chromarods were then developed in hexane – diethyl ether – formic acid (47.9:21.2:0.06, v/v/v) for 33 min and after drying were partially scanned for neutral lipid classes (diacylglycerol and TAG, free fatty acids, and sterols). Finally, Chromarods were developed in dichloromethane–methanol–water (42.2:25.3:2.5, v/v/v) for a further 33 min and after drying the entire rod was scanned for phospholipids. Rods were cleaned using the blank and origin scan procedures outlined in the Iatroscan instruction manual and stored in a dessicator when not in use.

Lipid compounds in individual larvae were identified by the position and the shape of the detector response curve (chromatograph) relative to known standards developed under identical conditions. Integration of the area under the chromatograph for each lipid class was used to estimate quantities (μg) of the classes present in a larva, and these estimates were corrected for recovery efficiency. Peak integration is somewhat subjective due to user-adjustable parameters in the T DATASCAN software. Two parameters that strongly affect peak integration are the maximum noise level and the tangent triggering percentage.

The maximum noise level is a percentage of the maximum signal amplitude. If the point to point change in amplitude is less than the designated percentage (in our case 0.2%) the signal is considered to be noise and is used to define the baseline. In most cases, this noise level provided an adequate fit. When the noise level did not provide an adequate fit the baseline was manually fit. This led to more accurate peak integrations.

The tangent triggering percentage determines which peaks are automatically detected and integrated. We set the tangent triggering percent at 0.2%, which was appropriate for automatic detection under most circumstances. Occasionally, dust particles on the chromarods resulted in narrow, spike-like peaks, which were not integrated despite their being identified as peaks by the T DATASCAN software.

We considered 0.03 μg as our limit of detection based on our calibrations, Lochmann et al. (1995), and Sebedio and Juaneda (1991). The calibration curves for tripalmitin (the synthetic standard for TAG) were linear to 0.05 μg. The minor extrapolation beyond this value maximized the information obtained from each larva without significantly exceeding the error around the lowest calibration value.

We corrected for larval size by dividing the square root of the lipid class measures by the DDW estimate of the larva. The square root transformation resulted in a higher coefficient of determination than any other transformation when transformed lipid concentration was regressed against DDW. The amount of TAG (the energy storage lipid) was used as the index of nutritional condition (Lochmann et al. 1995).

Morphometric condition

Total dry weight (TDW) was calculated by adding the absolute total of all measured lipids to the DDW. Standard lengths (SL) of videotaped larvae were measured to the nearest 0.1 mm using the OPTIMUS™ image analysis system (Bioscan, Seattle, Wash.; Miller et al. 1995). As regression analysis of the logarithmically transformed TDW and SL data showed evidence of allometric growth, we employed a Fulton-type index as another measure of condition (Fulton's $K = (TDW/SL^3) \times 100$). This condition index has proved useful to an

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number of investigators working with larval fish (Blaxter 1971; May 1971; Frank and McRuer 1989). However, as some marine fish larvae undergo a reduction in length and weight during periods of limited feeding (Ehrlich 1974), this index is best used in conjunction with other measures of condition.

Statistical analysis

Larvae collected from different locations were compared using analyses of variance. Trends in larval characteristics along the EZNET frontal transect were examined using linear regression analyses. Characteristics and indices of larval condition among samples were assessed using analyses of variance.

Results

Water mass physical structure

The three initial transects across the crest of Western Bank during the period 23–26 November revealed two distinctly different water masses and a third intermediate water mass (Fig. 2). Depth averaged measures showed relatively cool (<8.5°C), relatively fresh (<31.6 percent salinity units (psu)), low density (σ_t < 24.5) stratified water to the east of the bank crest that we refer to as the cold, fresh, water mass (CFW; Fig. 2). South of ~43°30′N there was warmer (>9.5°C), more saline (>32.2 psu), more dense (σ_t>24.8), and stratified slope water we refer to as the warm, salty water mass (WSW; Fig. 2). Water in the vicinity of the crest of Western Bank (~61°20′W, ~43°45′N) was intermediate in temperature (8.5–9.5°C), salinity (31.6–31.8 psu), and density (σ_t = 24.5–24.6), was well mixed, and we refer to it as the CW (Fig. 2). The temperature and salinity sections of the third transect (Fig. 3) show the tongue of stratified CFW (stations 5–9 and 14–17; Fig. 3) immediately southwest and northeast of the well-mixed CW (stations 10–13; Fig. 3) as it enveloped the CW from the east (Fig. 2). The stratified WSW (stations 1–4; Fig. 3) was located to the extreme southwest of the CFW along the 100-m isobath of the Scotian Shelf continental slope. The temperature difference between the CFW and the CW nearly compensated for the density difference between the water masses that would result from salinity differences alone, and thus, the density gradient between the two water masses was not strong (Fig. 3*b*).

The tongue of CFW intruding from the east at the beginning of the study continued to do so over the following week. Griffin and Thompson (1996) describe how the assimilation model was used to reconstruct a series of synoptic views (Fig. 4) of sea surface temperature (SST) observations by computing the trajectories of the thermally sampled waters. The reconstruction shows the CW as an anticyclonic gyre rotating around the crest of Western Bank during the period 25 November – 3 December. The reconstruction is perfectly consistent with Sanderson's (1995)[2] analyses of the drifter cluster that show the CW as a strongly anticyclonic eddy with baroclinic structure at its fringes. It is at the edges of the eddy, between the CW and the CFW, where the thermohaline front was formed as the tongue of CFW wrapped clockwise around the CW from the east. The frontal region was located at approximately 43°40′N, 61°15′W during the 27–28 November period (Fig. 4). Errors associated with computing the positions of the

[2] Sanderson (1995; p. 6762) erroneously reports his analyses as over the period 5–11 November 1992 when his analyses actually cover the period 25 November to 1 December 1992.

Fig. 2. Bathymetric charts (60-, 100-, and 200-m isobaths) of Western Bank for the period 23–25 November 1992 showing 10–40 m depth-averaged (*a*) temperature (°C), (*b*) salinity (psu), and (*c*) density (σ_t) isopleths, each showing the approximate locations of well-mixed crest water (CW), the stratified cold, fresh water (CFW), and the stratified warm salty water (WSW). Data are derived from the initial CTD survey in Fig. 1.

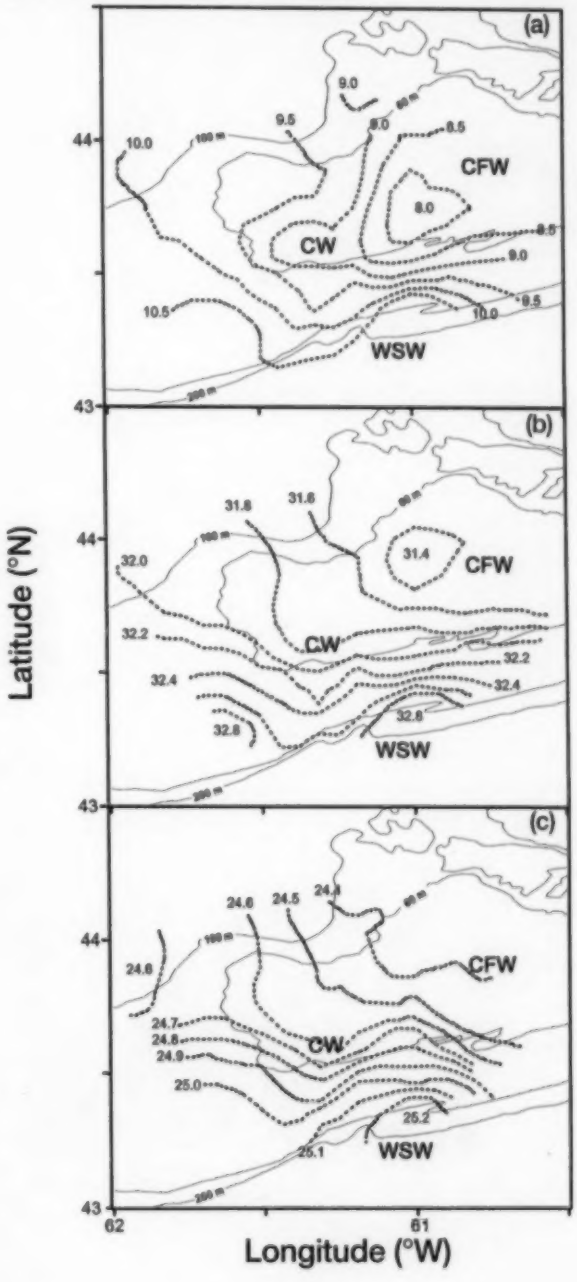

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Fig. 3. (*a*) Bathymetric chart (60-, 80-, 100-, and 200 m isobaths) of Western Bank showing locations of specific CTD stations from the initial CTD survey in Fig. 1 that were used to construct (*b*) density (σ_t), (*c*) temperature (°C); and (*d*) salinity (psu) sections across the crest of the bank, each showing the approximate locations of well-mixed crest water (CW), the stratified cold, fresh water (CFW), and the stratified warm salty water (WSW).

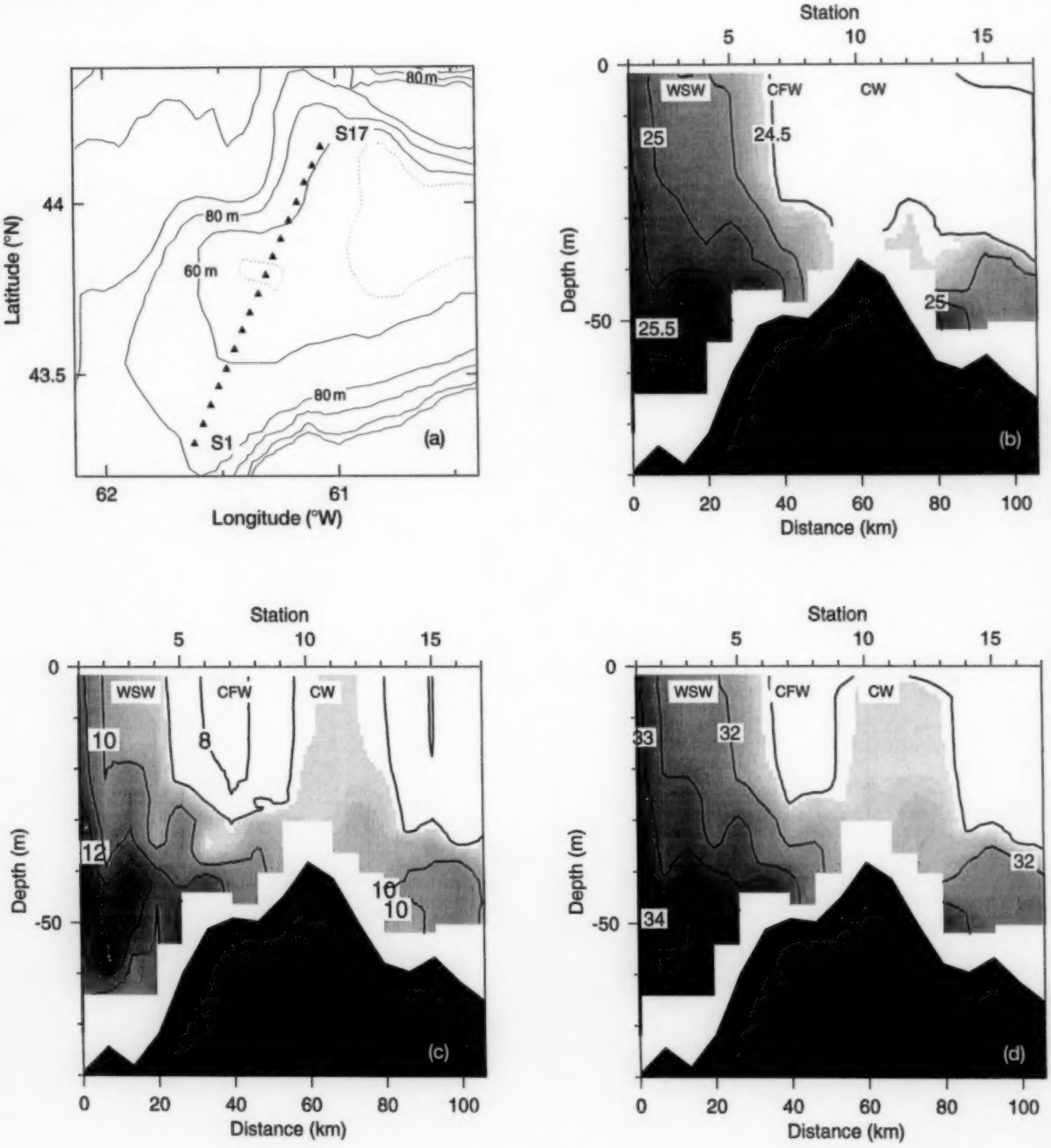

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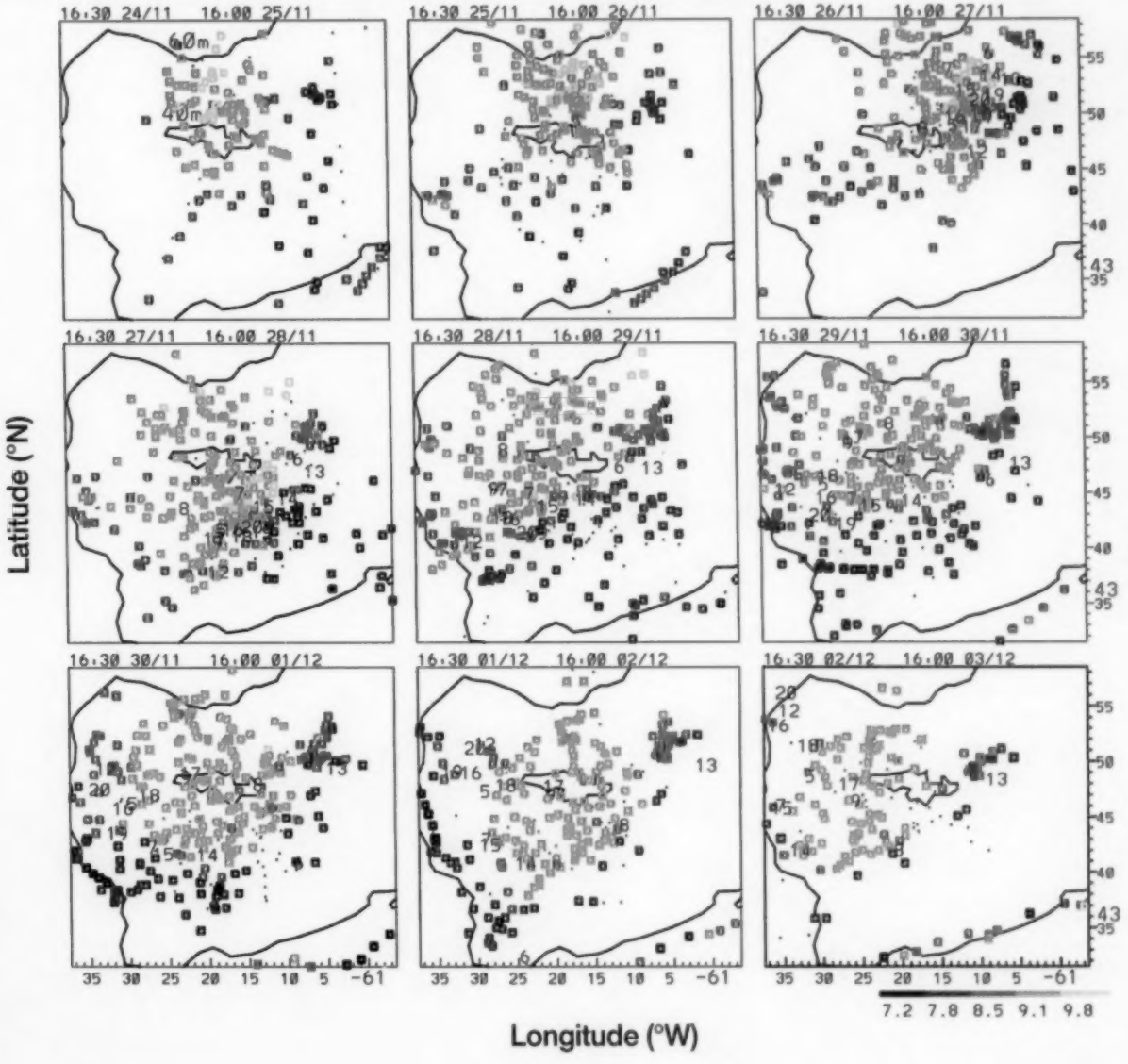
Fig. 4. Bathymetric charts (40- and 60-m isobaths) near the crest of Western Bank showing the evolution of the sea surface temperature (SST) fields computed using the flow fields provided by the data assimilative model of Griffin and Thompson (1996). Each panel shows the positions at the end of each 24-h period (UTC) noted at the top of each panel (hour:minute, day/month) of waters sampled by the ship and current meters during the preceding and succeeding 50 h. SST at the time of sampling is grey-scaled from cold (7.2°C, dark) to warm (9.8°C, light). Drifter positions at the end of each period are noted by their individual numbers as detailed in Fig. 5. Note how the drifters converge in panels 4 and 5 (27–29 November) in the frontal region between the warmer CW (light grey-scale) and the colder CFW (darker grey-scale) water masses. A more comprehensive analysis of these data are found in Plates 1 and 3 of Griffin and Thompson (1996).



SST samples (Fig. 4; observations from several days) and the large averaging interval (~30 min) for the SST data reduce the sharpness of the front in this figure. The front could actually be visually observed at the surface as the ship crossed.

Drifter trajectories

Drifter trajectories provided information regarding the persistence and nature of the frontal feature (Figs. 4 and 5) We observed the basic gyral structure of the CW rotating near the bank crest while a tongue of CFW wrapped around it from the east over an 8-day period (25 November – 3 December; Fig. 5). The drifter data indicated that the gyral structure at the crest and the frontal region became more irregular during, and subsequent to, the storm period that commenced on 3 December when the

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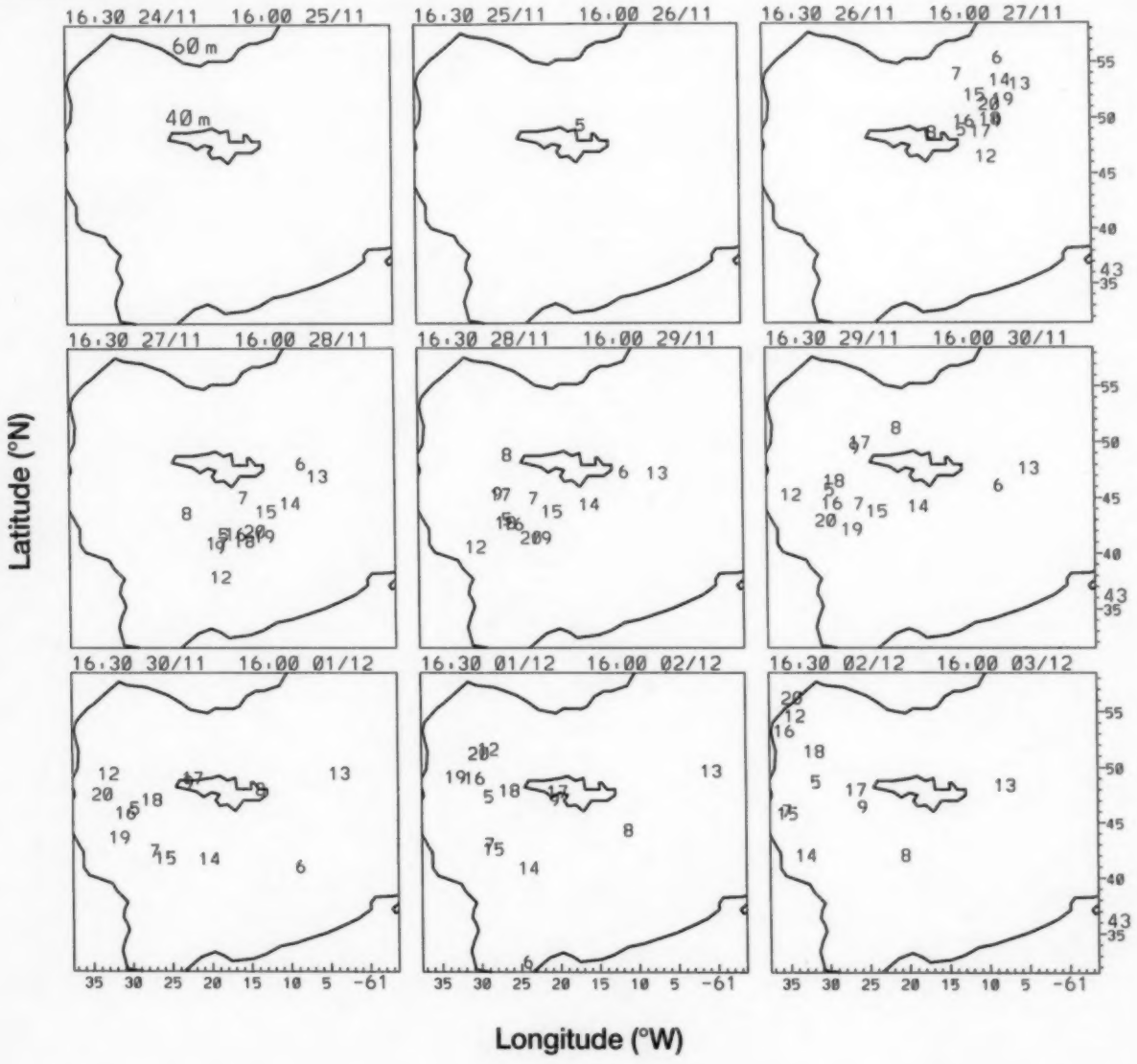
Fig. 5. Bathymetric charts (40- and 60-m isobaths) near the crest of Western Bank showing the daily sequence of 14 drifter positions. Drifter positions at the end of the time period concerned (UTC at top right of each panel; hour:minute, day/month) are indicated by their individual numbers 5–9 and 12–20.



water mass became stretched and distorted (Sanderson 1995). However, drifter trajectories from 26 November to 3 December provide evidence not only of the gyrelike structure, but that the frontal region was convergent. Drifter 5 was deployed near the crest of the bank in the CW (Fig. 5). The other 13 drifters, also deployed in the CW, were evenly spaced around drifter 5 by 04:30, 27 November UTC. Nine of the 14 drifters converged (Figs. 5 and 6 and see Sanderson 1995, Fig. 13) with one or more other drifters. This degree of convergence is much more than could be expected from random motion but insufficient to give a clear picture of the complexities of the convergence process. Note that between 27 and 29 November the drifters converged in the frontal region between the warmer CW (light grey-scale, Fig. 4) and the cooler CFW (darker grey-scale). Sanderson (1995) shows the drifter motion to be anything but random but noted that there were local zones of convergence or divergence and shear evident in the residual flow fields derived from the drifter data. Figure 6 shows how drifter pairs converged over periods ranging from 2 to 3 days and remained together for periods of 10–30 h before beginning to diverge. The velocity shear across the front is evident from the trajectory of drifter 6, which spent several days in the CW (Fig. 5; 27–30 November) before crossing the front (Fig. 5; 30 November – 1 December), to be swept quickly to the southwest

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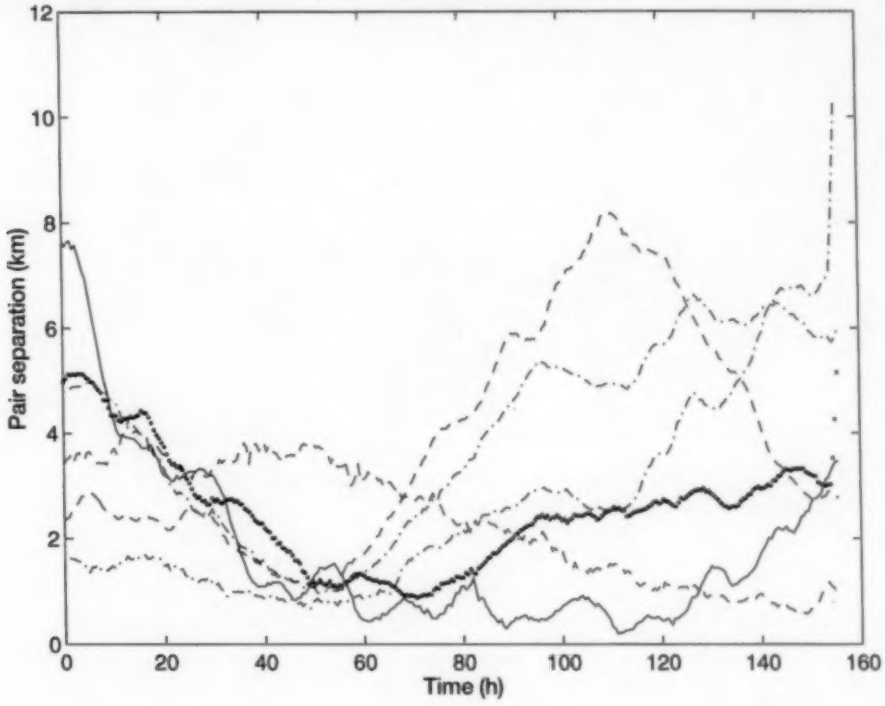
Fig. 6. Separation of those pairs of drifters shown in Fig. 5 that converged at some time between 04:30 on 27 November and 16:00 on 3 December UTC. Pairs are 17 and 9; 5 and 18; 18 and 16; 5 and 16; 20 and 19; and 7 and 15.



(Fig. 5; 1–2 December). Conversely, drifter 12, which had entered CFW (Fig. 5; 27–28 November), moved rapidly west and then converged toward the front (28–30 November) to finally re-cross the front and return to the CW (Fig. 5; 30 November – 2 December).

Water mass biological structure

Bongo net collections during the period 23–26 November indicated that cod larvae were present almost exclusively within the CW (Fig. 7). We used the hydrographic data and results above to classify the bongo and EZNET samples with respect to water mass. Samples collected in well-mixed, generally isothermal (to 25 m or more) water, with SST > 8.8°C were classified as CW samples. Those collected in stratified water with SST < 8.8°C were classified as frontal region samples. Samples collected in water that was isothermal to ~20 m, with SST < 8.0°C were classified as CFW samples. Samples were rarely collected in WSW and are not considered in these analyses.

The frontal region and adjacent water masses

Examples of EZNET deployments in the well-mixed CW and in the stratified frontal region show how the larval abundances were typically higher in the frontal region than in the CW, though similarly distributed through the water column and centred between 10 and 15 m (Fig. 8). The abundance of larval cod in the CW between 25 November and 3 December averaged 52 ± 33 larvae·100 m^{-3} (mean ± SD) while the abundance in the frontal region during the same period averaged 174 ± 40 larvae·100 m^{-3} (Table 1). Abundance estimates in both water masses were significantly higher (5- to 20-fold; Table 1) than those in the CFW (9 ± 12 larvae·100 m^{-3}). Coincidentally, zooplankton wet biomass was also significantly higher in the CW and in the frontal region than in the CFW (Table 1). Cod larvae from the CW and from the frontal region were marginally longer but not significantly heavier than larvae collected in the CFW (Table 1). As larval abundances in the CFW were systematically low (only nine larvae from CFW were available for morphometric and lipid analyses; see also Fig. 7) the significance of differences in length between the larvae collected there and in the other locations must be interpreted cautiously.

The TUBSS transect showed that the frontal region was several kilometres wide (Fig. 9). Relative to the CW, the CFW was cooler by ~1.5°C and fresher by about ~0.6 psu (Figs. 9*a* and 9*b*). Density differences were small over the same distance (Fig. 9*c*), consistent with the earlier mapping of the density field (Fig. 3*b*). Relative to the CW, the chlorophyll concentration was slightly lower in the frontal region and decreased further from ~7.0 mg·L^{-1} in the CW to <6.5 mg·L^{-1} in the CFW (Fig. 9*d*). Plankton abundances in the 231–314 µm equivalent spherical diameter (ESD) size range were also elevated to ~16–18·L^{-1} in the frontal region relative to ~8–12·L^{-1} in the CW (Fig. 9*e*).

Temperature and salinity observations from the EZNET frontal transect showed that, at the time and sampling location, the frontal region was approximately 1–1.5 km wide (Fig. 10).

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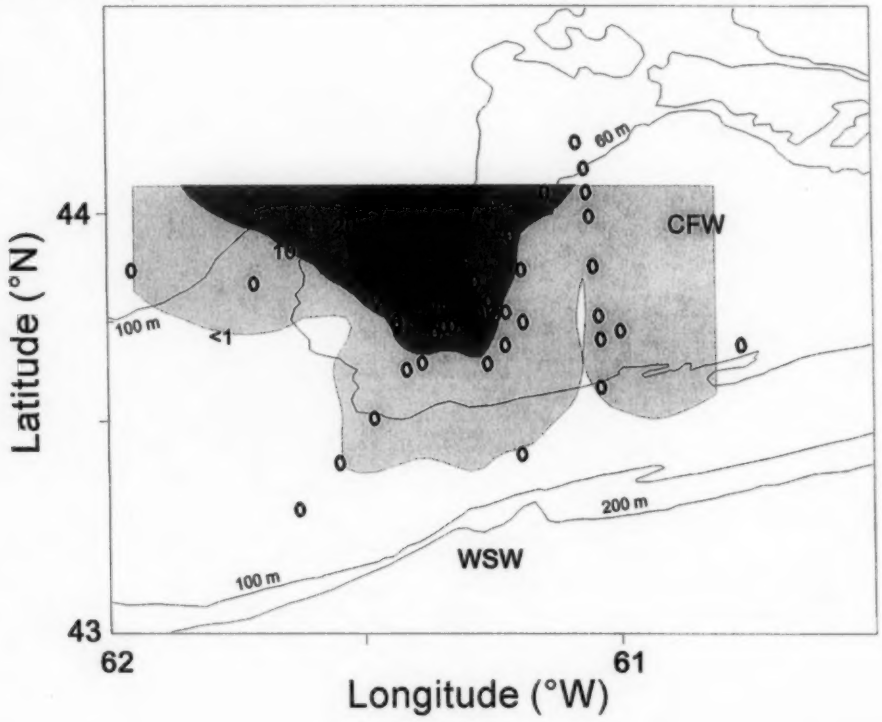
Fig. 7. Bathymetric chart (60-, 100-, and 200-m isobaths) of Western Bank showing grey-scaled depth-averaged abundance isopleths (larvae·100 m^{-3}) of cod larvae derived from bongo-net collections during the period of 23–26 November 1992 (open symbols). Grey-scale contours are in 10 larvae·100 m^{-3} intervals from <0 larvae·100 m^{-3} (open) to >50 larvae·100 m^{-3} (solid) and are associated with approximate locations of well-mixed crest water (CW), the stratified cold, fresh water (CFW), and the stratified warm salty water (WSW).



Temperature and salinity at 12.5-m depth decreased by ~1°C and ~0.35 psu respectively over a horizontal distance of 1.5 km from the well-mixed CW to the more stratified frontal region. Near the frontal region but still within the CW (nets 1–5; Fig. 10*d*), larval cod abundance was at or above the upper estimates typically observed in the CW as reported above (Table 1). However, larval abundance estimates virtually doubled over a distance of about 1 km (nets 4 and 5 compared with nets 7 and 8; Fig. 10*d*). The decreased larval abundance estimate for net 10 (Fig. 10*d*) reflects the change in depth as the BIONESS was removed from the water. Abundance estimates of plankton in two size-classes, 231–314 μm ESD (geometric mean 272 μm ESD) and 485–571 μm ESD (geometric mean 529 μm ESD) approximately doubled from the CW into the frontal region (Fig. 10*e*) while light attenuation decreased (Fig. 10*f*). Light attenuation, as measured by the OPC, is an indication of water colour (e.g., chlorophyll) and (or) the abundance of plankton below the resolution of the OPC (<231 μm ESD). We interpret the decrease in attenuation as an indication that phytoplankton (chlorophyll) abundance decreased in the frontal region, an observation that is consistent with fluorometer measurements collected during the TUBSS frontal transect (Fig. 9*d*).

Cod larvae collected in different nets during the EZNET frontal transect had similar average SL, TDW, and feeding ratio (Figs., 11*a*–11*c*, Table 2). Sample size for each net ranged from 8 to 10 larvae·net^{-1}. Average Fulton's K values were also similar for larvae from different nets (Fig. 11*d*, Table 2). Average TAG concentrations appeared higher in the frontal region than in the CW, but the differences among nets were not significant (Fig. 11*e*, Table 2). TAG concentrations of larvae from nets 7 to 10 in the frontal region were more variable than TAG concentrations from the other nets in the CW. The high variability of TAG concentrations in larvae in those nets was not due to the influence of one or two larvae with anomalously high TAG concentration (discussed below). Spatial trends were examined using linear regression of each larval characteristic or condition index against distance along the transect. No significant trends were found (i.e., regression slopes were not significantly different from zero at $\alpha = 0.05$). Analyses of variance showed no significant differences between the CW larvae (nets 1–5 combined) and frontal region larvae (nets 6–10 combined) for any characteristic or condition index (Table 2). It is possible that, by including larvae collected in net 6, which clearly sampled both the CW and the frontal region (Fig. 10), our analysis was unduly biased, and therefore, real differences between larvae from each water mass, particularly in the TAG index, could not be detected. However, no significant differences ($p = 0.064$) were detected when we repeated the analysis using only those samples

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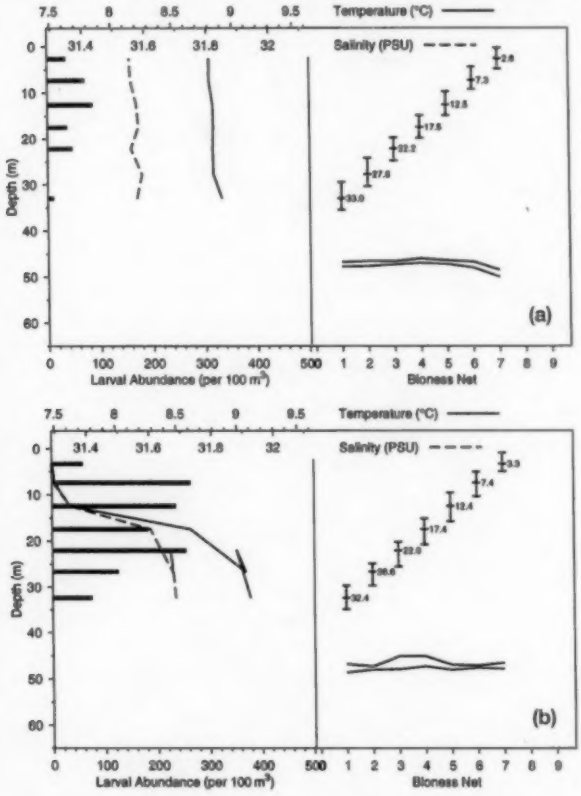
Fig. 8. Vertical profiles of larval cod abundance (larvae·100 m^{-3}, horizontal histograms), temperature (7.5–9.5°C, solid line) and salinity (31.4–32 psu, broken line) in the left panels, and bottom depth (minimum and maximum, solid lines) and sampling depth interval (minimum, maximum, and average) for each net in the right panels for EZNET (BIONESS) deployments in (*a*) the well-mixed crest water (CW) and in (*b*) the stratified frontal region.



collected in nets 1–5 (clearly in the CW) and those collected in nets 7–10 (clearly in the frontal region).

To further examine the variation in larval condition and to expand temporal and spatial resolution and sample size, we included all larvae available from samples in the CW and from those in the frontal region that were collected between 25 November and 3 December (>1000 larvae) in an analyses of variance (Table 3). For reference purposes, statistics for the nine larvae from the CFW were also tabulated but were not included in the analyses of variance. There was no significant difference in the TAG index, nor in the Fulton's *K* index between larvae from the CW and those from the frontal region (Table 3). However, feeding ratio was significantly higher for larvae from the CW than for larvae from the frontal region. As feeding periodicities have been noted in cod larvae (Kane 1984; McLaren and Avendano 1995), our inclusion of all cod larvae, regardless of collection time, may account for the difference in feeding ratio. Fifty-two of the 113 larvae analysed for gut contents from the CW were collected during the day, as were all of the larvae analysed for gut contents from the frontal region. When we repeated the analysis using daylight collections only, the feeding ratio showed no significant difference (Table 3).

Discussion

This study demonstrates that, relative to adjacent water masses and at scales of kilometres and less, larval cod can be as high as 20-fold more abundant in a narrow frontal region located between well-mixed CW and CFW on the Scotian Shelf. A number of processes may lead to such an observation. A simple explanation might be feeding-related differential survival of larvae among water masses (i.e., higher abundances of cod larvae are observed in the frontal region because larvae elsewhere are more food limited and are therefore dying at a faster rate). McLaren and Avendano (1995) showed that 3–6 mm cod larvae on Western Bank fed on plankton ranging from 80–480 μm ESD. Although the mesh size of the plankton nets (333 μm) used in this study and the lower limit of resolution for the OPC (231 μm) do not cover the entire feeding range, the 231-314 μm size-class was certainly well within the size range of prey consumed by larval cod in the region at the time of the study. The elevated levels of plankton in this size range in the frontal region are consistent with the resident larvae being provided with a relatively enhanced (more abundant) prey field. A logical consequence of enhanced feeding is improved condition and, thus, improved growth and survival in the frontal region.

Although the number of prey items in the gut is not an indication of feeding rate (Munk 1995), the index has been effectively used to quantify feeding success (Powell et al. 1990; Canino et al. 1991). The average number of prey items we observed in the guts of the larvae examined indicated that larvae from the CW and from the frontal region were feeding successfully but not differentially. This is consistent with the fact that neither the average of Fulton's *K* nor the average TAG concentration differed significantly between larvae from the CW and the frontal region, suggesting that condition and survival probability were similar in both water masses. We thus conclude that the elevated abundance of cod larvae in the frontal region did not stem directly from differential survival that resulted from differential condition and (or) food availability.

An alternative, and equally simple, explanation for the elevated abundance of cod larvae in the frontal region is convergence, whereby passive transport brings larvae into the frontal region. Depending upon variations in hydrography and the nutritional condition of the cod larvae (see Sclafani et al. 1993), the buoyant nature of larvae could allow them to remain at a convergent front while the water that carried them into the frontal region was subducted and advected away. The drifter trajectories shown here and those of Sanderson (1995) indicate that the front was convergent. As we have no evidence to suggest that the elevated abundance of larvae in the frontal region was due to differentials in the biological processes we considered, we conclude that the simplest explanation is that a physical process was responsible for the enhanced larval abundance in the frontal region. This is consistent with McLaren and Avendano (1995) who concluded that enhanced zooplankton abundance on the bank did not result from enhanced in situ

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Fig. 9. Sections of (*a*) temperature (°C); (*b*) salinity (psu); (*c*) density (σ_t); (*d*) fluorometer (mg chlorophyll $a \cdot L^{-1}$); and (*e*) 231–314 μm ESD plankton abundance (particles·L^{-1}) derived from the TUBSS transect at the front (~43°40′N, 61°14′W on 29 November; see Fig. 1*b* for location). The frontal region (FRONT, ~5–7 km) was located between stratified cold, fresh water (CFW, ~0–5 km) and the well-mixed crest water (CW, ~7–12 km). The TUBSS tow profile is shown in the top panel (*a*).

Table 1. Mean (±SD) characteristics of larval cod and zooplankton collections from crest water (CW), the frontal region (Front), and cold, fresh water (CFW) during the period 25 November – 3 December 1992 on the Western Bank.

	Location		
Characteristic	CW	Front	CFW
Larval cod abundance (no.·100 m^{-3})	52±33 (42)*b*	174±40 (10)*a*	9±12 (11)*c*
Zooplankton wet biomass ($g \cdot m^{-3}$)	0.22±0.11 (42)*a*	0.29±0.12 (10)*a*	0.12±0.13 (11)*b*
Standard larval length (mm)	4.75±0.95 (794)*a*	4.86±0.84 (294)*a*	4.01±0.56 (9)*b*
Larval total dry weight (μg)	103.2±85 (747)*a*	107.5±84.4 (284)*a*	63.4±36.1 (9)*a*

Note: Values followed by the same letter are not significantly different ($\alpha = 0.05$) according to Duncan's multiple-range test. Sample size (number of deployments or larvae) for each location and characteristic is given in parentheses.

productivity but rather from physical concentration within the gyrelike CW.

In summary, larvae in the stratified frontal region experienced an enhanced prey field, but they were not in significantly better condition than larvae from the CW. This appears contrary to the hypothesis that positive correlations between larvae and their prey result in improved condition and survival. While we observed no difference between cod larvae from stratified and well-mixed sites at small scales, Buckley and Lough (1987) showed that, at somewhat larger scales, haddock (*Melanogrammus aeglefinus*) larvae on Georges Bank in stratified water masses were in better condition (index based on RNA/DNA ratio) than larvae in well-mixed water masses.

It is possible that larvae did not reside in the frontal region long enough to be distinguished from larvae elsewhere on the basis of their condition alone. The seemingly contrary nature of our results led us to explore this possibility. We used the drifter trajectories in and around the frontal region to estimate the range of time a cod larva might reside in the enhanced food environment of the frontal region, and we examined the estimates in relation to estimates of the latency period for the condition indices we used.

To a first approximation, drifters, larvae, and prey items will all be aggregated by the evolving convergent flow field and subsequently dispersed at the same rate. Hence, one estimate of the length of time that the larvae and prey are both aggregated is simply given by the length of the period (2–3 days) that pairs of converging drifters took to converge, since this is an estimate of the lifetime of the structure drawing them together. In other words, if one drifter is approaching another, then at least one of them is in a region where elevated abundances of buoyant material are likely to be found. A more conservative estimation is to measure the time that convergent drifters remained aggregated, which ranged from 0 to 30 h. The drifter trajectories also provide an estimate of the concentration factor achieved by the convergence and subduction process. Assuming that the drifters converged to a line rather than to a point, then the ratio of the initial separation to the final separation is an estimate of the increase of the number of particles per unit area. Figure 6 shows this factor to be of the order 5. Hence, we conclude that larvae sampled in the frontal region may have experienced a prey field that was up to fivefold more concentrated than in the adjacent waters for periods as long as 3 days. Given the paucity of data, we are reluctant to estimate the uncertainty of these estimates but note that larvae and zooplankton in the frontal region were observed to be at least twice as abundant as in the CW.

Ferron and Leggett (1994, p. 252) defined the latency of a condition index as "the time required for a given change in food availability to be reflected as a significant change in the particular index of condition used." Their summary suggests that the latency period for morphological and lipid-based indices are days and hours to days, respectively (see Ferron and Leggett 1994, Table 2, p. 284). Calculations, based on optimal feeding rates, prey lipid composition, and energy content, and oxygen consumption rates suggest that it could take between 18 h and 3.3 days for a cod larvae to increase its TAG level from 0.0025 to 0.0075 $(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW (see Appendix for details of the assumptions and calculations). In our laboratory experiments (Lochmann et al. 1995), starved cod larvae lost an average of 0.005 $(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW over a 6-day period, and the average TAG concentration of cod larvae fed at 1000 prey·L^{-1} increased from 0.005 to 0.015 $(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW over a 6-day period. Together, all of these estimates suggest that it could take from 18 h to 6 days for the TAG concentration of a larva to increase from the lowest average level observed in the CW during the frontal transect (0.0020 $(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW; Fig. 11, net 4) to the highest average level observed in the frontal region (0.0082 $(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW; Fig. 11, net 9).

The 18-h to 6-day time scale for changes in our condition measures and the estimated time that a larva might have experienced an enhanced prey environment (as high as 3 days) are of the same order. All larvae collected from the frontal region may not have resided in the region long enough to have improved in condition to a point where differences between groups from there and elsewhere became apparent. Some larvae may have benefitted from a longer residence in the frontal region, which is consistent with the high TAG concentration variances observed in nets 7–10 of the EZNET frontal transect (Fig. 10*e*). If larvae in relatively poor condition from outside the frontal region were mixed with larvae in relatively good condition within the frontal region, then sample variances in the frontal region would be expected to be higher than elsewhere,

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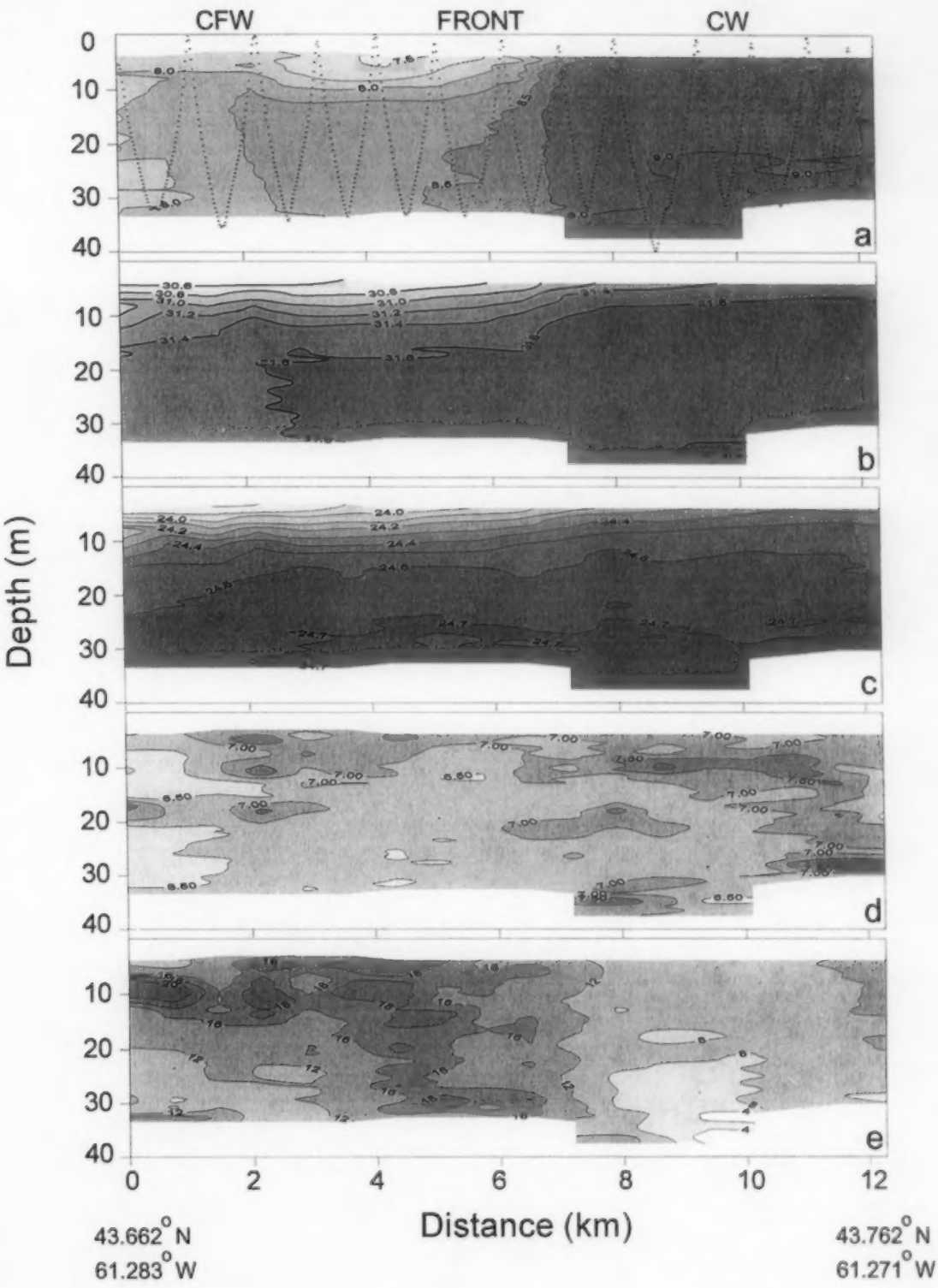

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Fig. 10. (*a*) Sampling depth (m), (*b*) temperature (°C), (*c*) salinity (psu), (*d*) larval cod abundance (larvae·100 m^{-3}), (*e*) 272 and 529 µm geometric mean ESD zooplankton abundance (particles·L^{-1}), and (*f*) light attenuation (relative) derived from the frontal transect with the EZNET towed at a fixed depth (~12.5 m) normal to the front on the Western Bank (see Fig. 1*b* for chart location). Larval cod abundances are reported at the midpoint of each sequential net tow. Net numbers and locations of closures are noted by vertical lines on the depth profile in (*a*).

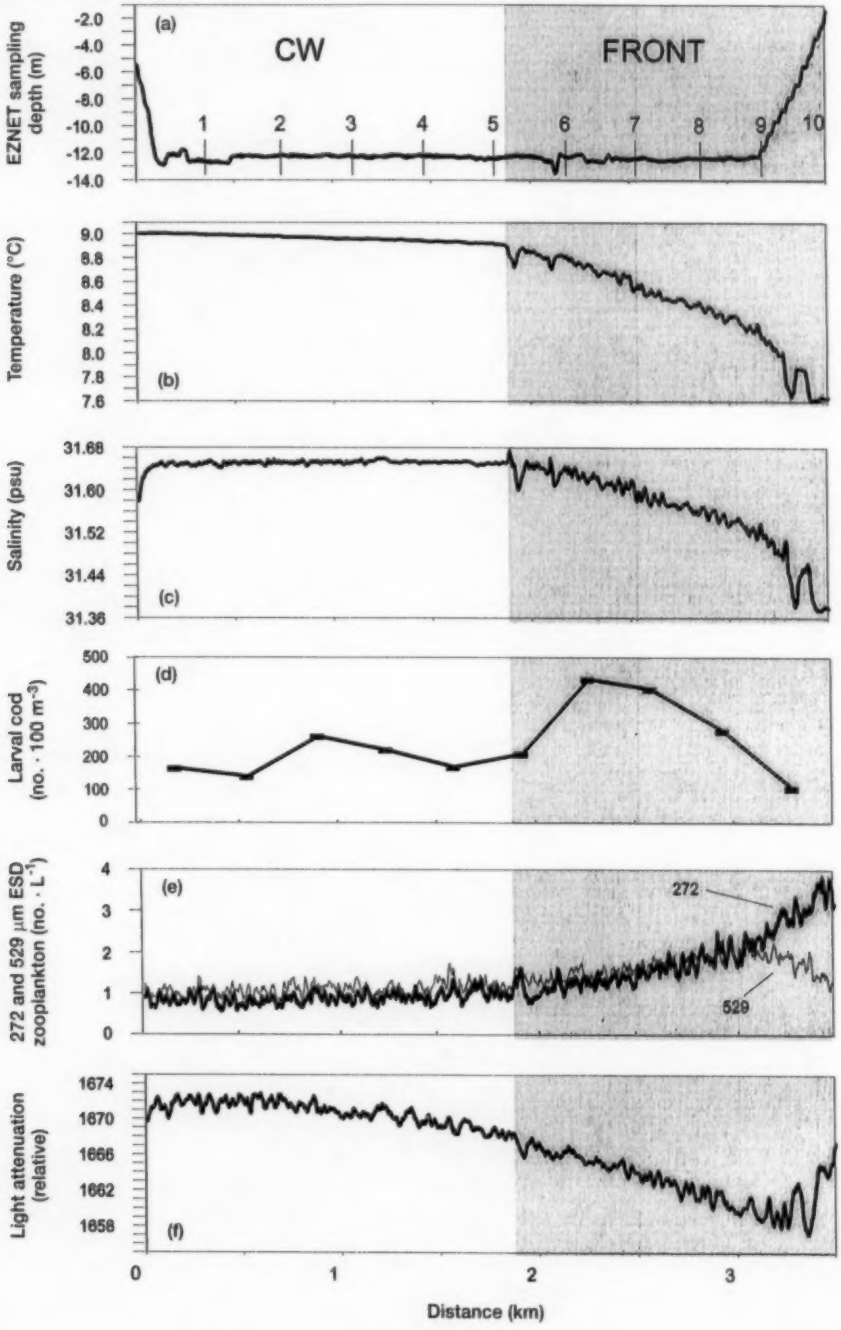

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Fig. 11. Average (±2 SE) of (*a*) standard length (mm); (*b*) total dry weight (μg); (*c*) feeding ratio; (*d*) Fulton's *K*; and (*e*) triacylglycerol (TAG) concentration ($(\mu g)^{1/2} \cdot \mu g^{-1}$ DDW) of cod larvae collected in each of 10 different nets along the EZNET transect normal to the front shown in Fig. 10 (see Fig. 1*b* for chart location).

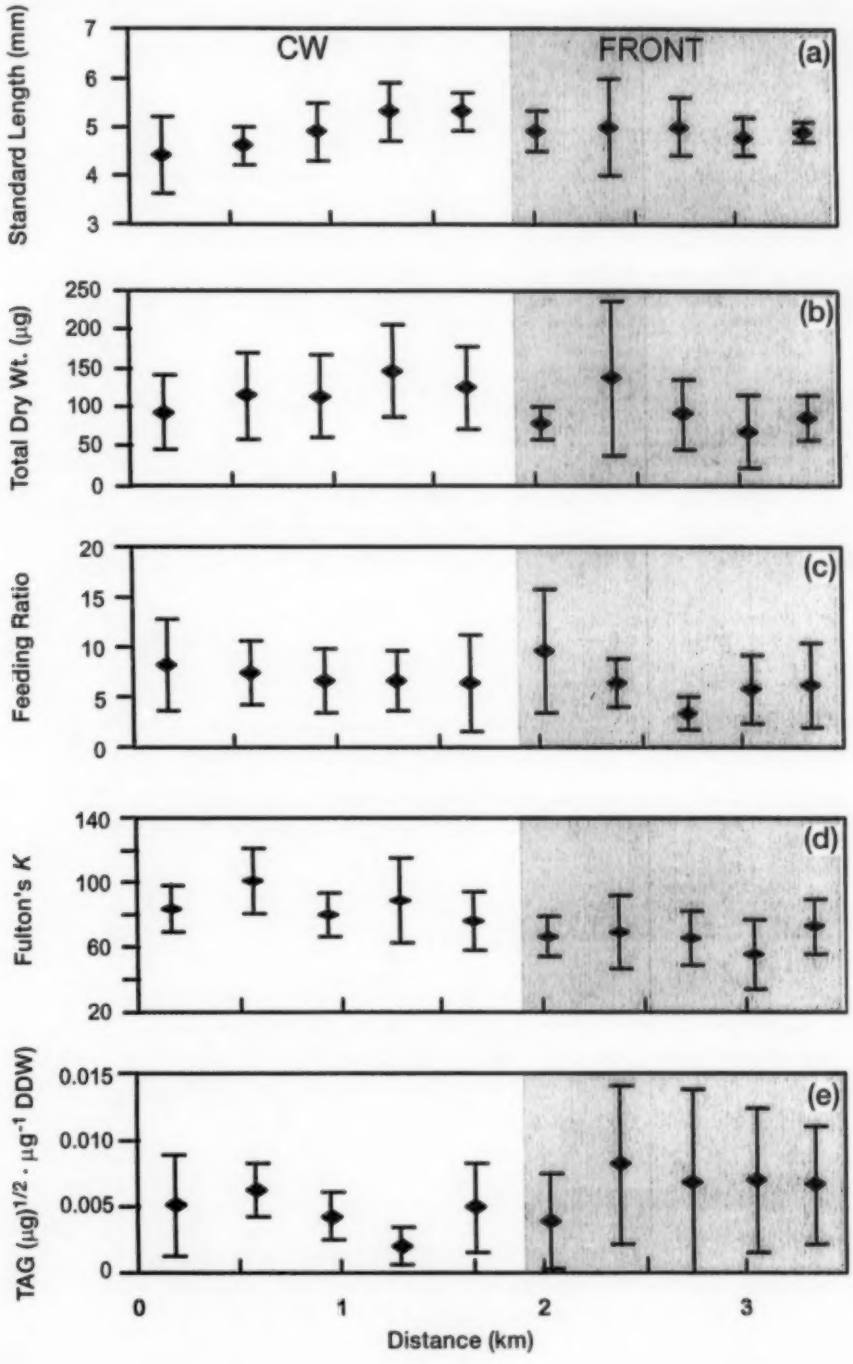

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Table 2. Mean (±SD) characteristics of larvae from nets 1–5 deployed in crest water (CW) and nets 6–10 deployed in the frontal region (Front) during the EZNET frontal transect.

Characteristic	Location	
	CW	Front
Standard length (mm)	4.9±0.9 (48)	4.9±0.8 (44)
Total dry weight (μg)	118.1±79.4 (46)	92.8±75.9 (43)
Triacylglycerol ($(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW)	0.005±0.004 (46)	0.006±0.007 (43)
Fulton's *K*	85.9±28.6 (46)	77.2±75.5 (43)
Feeding ratio	7.0±5.9 (50)	6.1±6.0 (50)

Note: Means were not significantly different according to Duncan's multiple-range test. Sample size for each location and characteristic is given in parentheses.

Table 3. Mean (±SD) condition of larvae from crest water (CW), the frontal region (Front), and cold, fresh water (CFW).

Condition index	Location		
	CW	Front	CFW
Triacylglycerol ($(\mu g)^{1/3} \cdot \mu g^{-1}$ DDW)	0.006±0.006 (758)*a*	0.005±0.006 (288)*a*	0.014±0.009 (9)
Fulton's *K*	86±43 (745)*a*	84±60 (284)*a*	89±23 (9)
Feeding ratio (day and night)	8.9±6.0 (113)*a*	6.5±5.9 (100)*b*	17.0±8.4 (8)
Feeding ratio (day samples only)	6.8±4.0 (52)*a*	6.5±5.9 (100)*a*	

Note: Values followed by the same letter are not significantly different ($\alpha = 0.05$) according to Duncan's multiple-range test. Larvae from CFW were not included in the statistical analyses. Sample size of larvae for each location and condition index is given in parentheses.

though the sample means would not necessarily be statistically different. We observed larvae in equally poor condition in the CW and in the frontal region. However, the higher variances in nets 7–10 of the EZNET frontal transect were due to several larvae from each of those nets having high TAG concentrations, and not due to just one or two outliers. The overall average TAG index for all CW and frontal larvae (Fig. 11, all nets) was 0.0054 and 33% of the larvae (15 of 46) from the CW were in better than average condition while 44% of the larvae (19 of 43) from the frontal region were in better than average condition. In retrospect, condition indices that have shorter latencies, such as RNA/DNA ratios or digestive enzyme activity, may be a more appropriate index when addressing questions at the times scales of the physical processes occurring in frontal regions.

Here we provide a conceptual model of the processes occurring in the frontal region that is consistent with the above results. Figure 12 summarizes how the CW, intermediate in temperature and salinity between the CFW and the WSW, rotated anticyclonically as a slab-like water mass near the crest of Western Bank (see also Sanderson 1995; Griffin and Thompson 1996). A tongue of CFW, impinging from the east, wrapped around the rotating CW creating a convergent frontal region at the margin between the CW and the CFW. Cod larvae from the CW (and presumably spawned there; see Frank et al. 1994; Miller et al. 1995) and larvae from CFW (which we noted were very few in number) were retained in the CW and (or) concentrated in the frontal region where they experienced an enhanced prey field. The frontal region included larvae that had spent varying amounts of time (hours to days) within the enhanced prey environment, resulting in our inability to reject the null hypothesis that larvae in the frontal region were in the same condition as larvae elsewhere. As the CW continued to rotate, larvae in the frontal region were either re-introduced to the CW inside the frontal region or dispersed outside the frontal region and advected from the region by west-south-west residual currents (Sanderson 1995, Griffin and Thompson 1996).

The global importance of gyrelike features and convergent fronts, which allow early stage fish to remain on or near banks, was first discussed by Iles and Sinclair (1982). Although simple geographic observations of persistently high larval abundance are insufficient to distinguish between physically driven retention and biologically driven differential mortality, our observations clearly suggest it is physically driven retention and not differential mortality (at least as approximated by condition indices) that is responsible for the high abundances of cod larvae within a relatively small area on the crest of Western Bank. The difference between these two mechanisms is important as they directly determine which measures are necessary for predictive purposes. However, retention in this area also seems to provide larvae with a more abundant and more appropriate prey field than elsewhere on the bank (McLaren and Avendano 1995). A high percentage of the drifters that converged in the frontal region were re-introduced into the CW, rather than being carried away from the bank crest in the CFW or the WSW. Larvae entrained into the frontal region and recirculated within this semiclosed system (the CW and the frontal region), probably benefitted from retention in a small region near the crest of the bank where the large-scale prey field is clearly enhanced relative to the entire bank.

The semiclosed system and convergent front we describe appears to have important life-history consequences for cod. We know this area is an important cod spawning region (Frank et al. 1994) and most recently an important spawning region in the October–December period (Miller et al. 1995) when the

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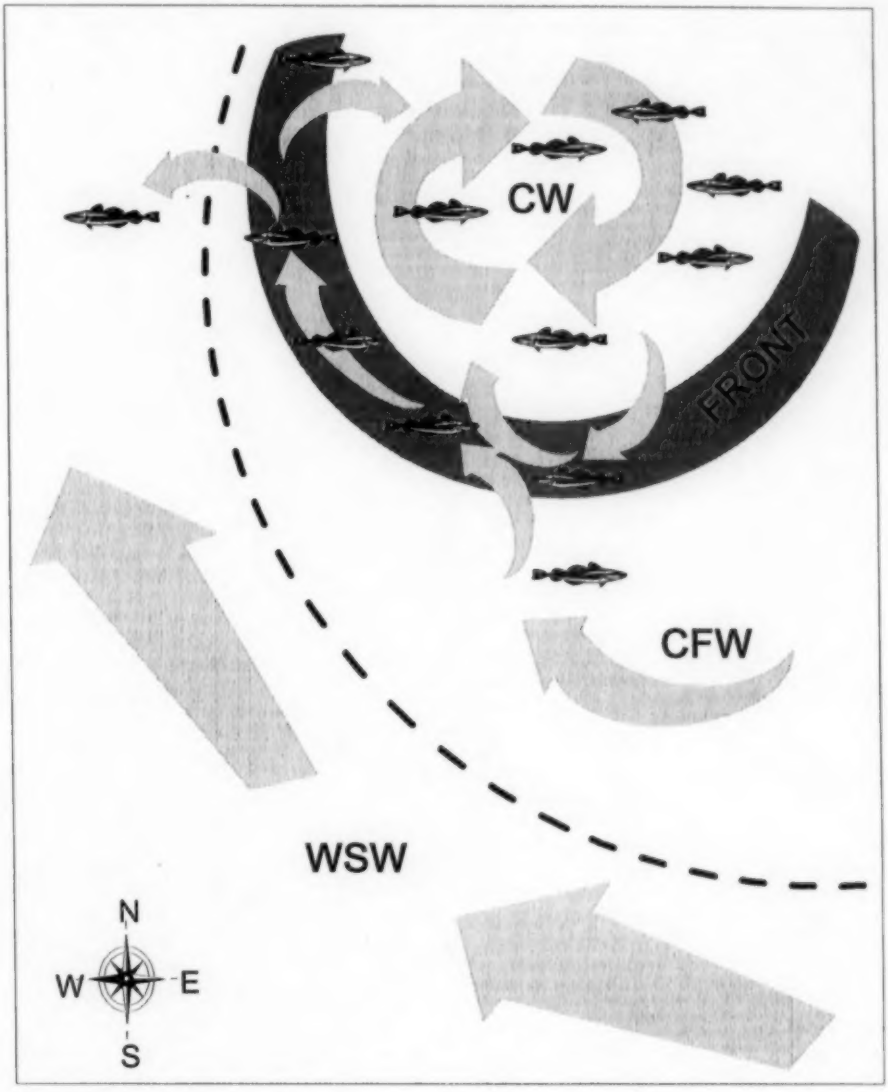
Fig. 12. Conceptualized model of the biophysical processes at the crest of Western Bank. Crest water (CW) rotates as an anticyclonic slab. Periodically, a tongue of cold, fresh water (CFW) is wrapped around the CW forming a frontal region between the two water masses. Cod larvae from CFW (rare) and CW (abundant) are entrained (converge) at the front where the prey field is enhanced. Larvae are transported in the frontal region to the west and north where they are either mixed back into the CW or out into the CFW or the warm salty water (WSW) flowing along the bank margin. Residual currents transport larvae outside the CW and the frontal region off the bank. Larvae mixed back into the CW are retained on the bank and may be repeatedly entrained into the frontal region.



prey field for larvae is regionally enhanced (McLaren et al. 1995). Thus, we hypothesize that interannual variability in the formation and stability of the gyrelike feature and the integrity of the frontal system may account for some of the recruitment variability observed in Western Bank cod. Alternatively, retention on the bank may simply maintain a spatial overlap between cod larvae and their prey. Observations of the abundance and condition distributions at the crest of Western Bank in years when retention occurs but when prey fields are less favourable should help ascertain which of the biological, physical, or biophysical processes are most significant in explaining and predicting recruitment variation in the region.

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Appendix

We considered a range of daily consumption rates for larval cod. Tilseth and Ellertsen (1984) provide a feeding rate of 1.5 nauplii·h^{-1}. As cod larvae are visual feeders (Ellertsen et al. 1980) and need a minimum light level for feeding we assumed a 12 h feeding period on the Scotian Shelf in November, leading to an estimate of 18 nauplii·day^{-1}. This a conservative estimate as cod larvae from the study area have been observed with as many as 29 nauplii per gut (McLaren and Avendano 1995).

Solberg and Tilseth (1984) report a caloric content of 1.7×10^{-6} kcal (1 kcal = 4.186 kJ) for a 0.3-µg nauplii, which is consistent with a caloric content of 1.5×10^{-6} kcal for a 0.3-µg nauplii on the basis of a proximate composition of 60% protein, 27% lipid, 10% ash, and 3% carbohydrate (Parsons et al. 1984). Assimilation efficiencies for cod larvae are estimated at between 40 and 50% (Solberg and Tilseth 1984). Using the maximum estimates of consumption (29 nauplii·day^{-1}), caloric content (1.7×10^{-6} kcal·nauplii^{-1}), and assimilation efficiency (50%), a cod larva might have 2.47×10^{-5} kcal·day^{-1} available to meet energy requirements.

Estimates of oxygen consumption rates for early cod larvae range from 2.0 µL O_2·mg^{-1}·h^{-1} (Solberg and Tilseth 1984) to 2.5 µL O_2·mg^{-1}·h^{-1} (I. Hunt Von Herbing, Department of Biology, Woods Hole Oceanographic Institute, Woods Hole, Mass., personal communication). Therefore, using a metabolic conversion of 4.6×10^{-6} kcal·µL^{-1} O_2·mg^{-1} an early cod larva weighing approximately 50 µg total dry weight (Lochmann et al. 1995) would have catabolic requirements ranging from 1.10×10^{-5} to 1.35×10^{-5} kcal·day^{-1}. Growth rates for cod larvae range from 5 to 7%·day^{-1} (Solberg and Tilseth 1984) and to 10%·day^{-1} (Buckley and Lough 1987). The energy content of cod larvae has been estimated to be approximately 5.0×10^{-6} kcal·µg^{-1} ash free dry weight (Solberg and Tilseth 1984). Therefore, anabolic requirements for a 50-µg cod larva would range from approximately 1.25×10^{-5} to 2.50×10^{-5} kcal·day^{-1}. Assuming an oxygen consumption rate of 2.0 µL O_2·mg^{-1}·h^{-1} and a growth rate of 5%·day^{-1} there would be a maximum excess of 1.2×10^{-6} kcal·day^{-1}.

According to the estimates of the proximate composition of copepods, one half of the available energy comes from lipids and the other half from proteins. We assumed that one half of the excess (storage) energy is in the form of lipids. Nine kilocalories are equivalent to one gram of lipid. Estimates of the proportion of lipid in the form of TAG range from 4% for *Calanus helgolandicus* (Lee et al. 1970) to 4–12% for *C. pacificus* (Hakanson 1984). However, wax esters, another energy storage product might be converted to TAG for storage in cod larvae. Wax esters make up 30% of the lipids in *C. helgolandicus* (Lee et al. 1970) and between 12 and 65% of lipids in *C. pacificus* (Hakanson 1984). If wax esters were not converted to TAG by cod larvae, and TAG made up only 4% of the excess lipids, then TAG would accumulate at a rate of 0.0027 µg TAG·day^{-1}. If 65% of lipids were wax esters and 12% of lipids were TAG, and wax esters were converted to TAG for storage in cod larvae, then TAG would accumulate at a rate of 0.0511 µg TAG·day^{-1}. Lipids make up approximately 30% of the total dry weight of early cod larvae (Lochmann et al. 1995), so a 50-µg cod larvae would have a DDW of 35 µg. If we correct the TAG accumulation rates for body size using the index defined in the main body of the text TAG would accumulate at between 0.0015 and 0.0065 (µg)$^{1/3}$·µg^{-1} DDW·day^{-1}. As we observed differences in TAG concentration between CW (0.0025 (µg)$^{1/3}$·µg^{-1} DDW·day^{-1}) and frontal (0.0075 (µg)$^{1/3}$·µg^{-1} DDW·day^{-1}) larvae, then we can estimate that it would take between 18 h and 3.3 days for a larva to increase its TAG concentration by 0.005 (µg)$^{1/3}$·µg^{-1} DDW·day^{-1}. This estimate is not only sensitive to the range of values for the variables used above but also to the proportion of energy requirements that are met by utilizing protein as opposed to lipids.

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Predation on fish by cormorants and pelicans in a cold-water river: a field and modeling study

Clayton E. Derby and James R. Lovvorn

Abstract: We studied diets of double-crested cormorants (*Phalacrocorax auritus*) and American white pelicans (*Pelecanus erythrorhynchos*) collected on the North Platte River, Wyoming, from May to August. Rainbow (*Oncorhychus mykiss*) and cutthroat (*O. clarki*) trout 10–16 cm long were stocked in late June – early July. Cormorants ate mainly suckers (*Catastomus* spp., 61% of numbers, 85% of fresh mass) before trout stocking; but trout consumed increased from 17% (12% of mass) in samples before stocking to 60% (82% of mass) after stocking. Pelicans ate mostly suckers (64% of numbers, 84% of mass) and minnows (*Rhinichthys cataractae*, *Pimephales promelas*; 21% of numbers, 1% of mass) throughout the study; trout were 1% (0.1% of mass) of the pelican diet before stocking, and 22% (14% of mass) after stocking. A bioenergetics model estimated that these birds consumed up to 80% of trout stocked in 1994. In August 1993, cormorants ate mostly trout (86% of numbers, 98% of mass), suggesting that many stocked trout survived until autumn. However, the much smaller fraction of trout eaten by cormorants in spring 1994 before stocking (mean 17% of numbers, 13% of mass) than after stocking (60% of numbers, 82% of mass) suggests that stocked trout declined substantially during winter. Because overwinter survival might have limited trout recruitment between years, it is unclear whether bird predation was additive or compensatory.

Résumé : Nous avons étudié les régimes alimentaires du cormoran à aigrettes (*Phalacrocorax auritus*) et du pélican blanc d'Amérique (*Pelecanus erythrorhynchos*) provenant de la rivière North Platte, au Wyoming, de mai à août. Des truites arc-en-ciel (*Oncorhynchus mykiss*) et des truites fardées (*O. clarki*) de 10–16 cm de long ont été ensemencées à la fin de juin et au début de juillet. Les cormorans consommaient principalement des catostomes (*Catostomus*, 61% en nombre, 85% en masse fraîche) avant l'ensemencement des truites; mais la consommation de truites a augmenté de 17% (12% en masse) dans les échantillons avant l'ensemencement à 60% (82% en masse) après cet ensemencement. Les pélicans ont consommé principalement des catostomes (64% en nombre, 84% en masse) et des cyprins (*Rhinichthys cataractae, Pimephales promelas;* 21% en nombre, 1% en masse) pendant toute la durée de l'étude; la truite constituait 1% (0,1% en masse) du régime alimentaire du pélican avant l'ensemencement, et 22% (14% en masse) après l'ensemencement. Un modèle bioénergétique a permis d'estimer que ces oiseaux ont consommé jusqu'à 80 % de la truite ensemencée en 1994. En août 1993, les cormorans ont consommé principalement de la truite (86% en nombre, 98% en masse), ce qui indiquerait que de nombreuses truites ensemencées ont survécu jusqu'à l'automne. Cependant, la fraction beaucoup plus petite de truites consommées par les cormorans au printemps de 1994 avant l'ensemencement (moyenne de 17% en nombre, 13% en masse) qu'après l'ensemencement (60% en nombre, 82% en masse) indiquerait que la truite ensemencée a décliné de manière substantielle au cours de l'hiver. Comme la survie en hiver a pu limiter le recrutement de la truite d'une année à l'autre, il n'est pas clair si la prédation par les oiseaux était additive ou compensatoire.
[Traduit par la Rédaction]

Introduction

Double-crested cormorants (*Phalacrocorax auritus*) and American white pelicans (*Pelecanus erythrorhynchos*) have increased in much of North America, including Wyoming (Sidle et al. 1985; Findholt 1988; Findholt and Diem 1988; Hatch 1995; see regional reviews in Nettleship and Duffy 1995). Growing numbers of these birds have raised concerns about possible impacts on fish populations, prompting proposals and enactment of laws to open hunting seasons on cormorants (Nettleship and Duffy 1995).

An important question is whether fish species consumed by cormorants and pelicans have sport or commercial value. Studies indicate that double-crested cormorants are highly opportunistic feeders. At lakes, estuaries, and rocky coasts, they often eat mainly benthic, slow-moving fish (Robertson 1974; Dunn 1975*a*; Ross 1976; Pilon et al. 1983; Craven and Lev 1987; Cummings 1987; Blackwell et al. 1995; Glahn et al. 1995). However, in other cases in similar environments, they feed more on faster moving fish in the water column, including trout (Ainley et al. 1981; Hobson et al. 1989; Ludwig et al. 1989; Campo et al. 1993; Ottenbacher et al. 1994; Modde et al. 1996). In an Irish river, great cormorants (*Phalacrocorax carbo*) ate many wild trout and both stocked and wild salmon smolts (Kennedy and Greer 1988; Warke and Day 1995), but we found no data on double-crested cormorants feeding in cold-water rivers. In inland lakes, American white pelicans are also highly opportunistic (Lingle and Sloan 1980; Anderson 1987; Findholt and Anderson 1995). Foraging only from the water surface, white pelicans usually do not eat many sport fish; although in a study in Yellowstone Lake, cutthroat trout (*Oncorhynchus clarki*) were their main prey (Davenport 1974). There are no published data on diets of American white pelicans collected in cold-water rivers.

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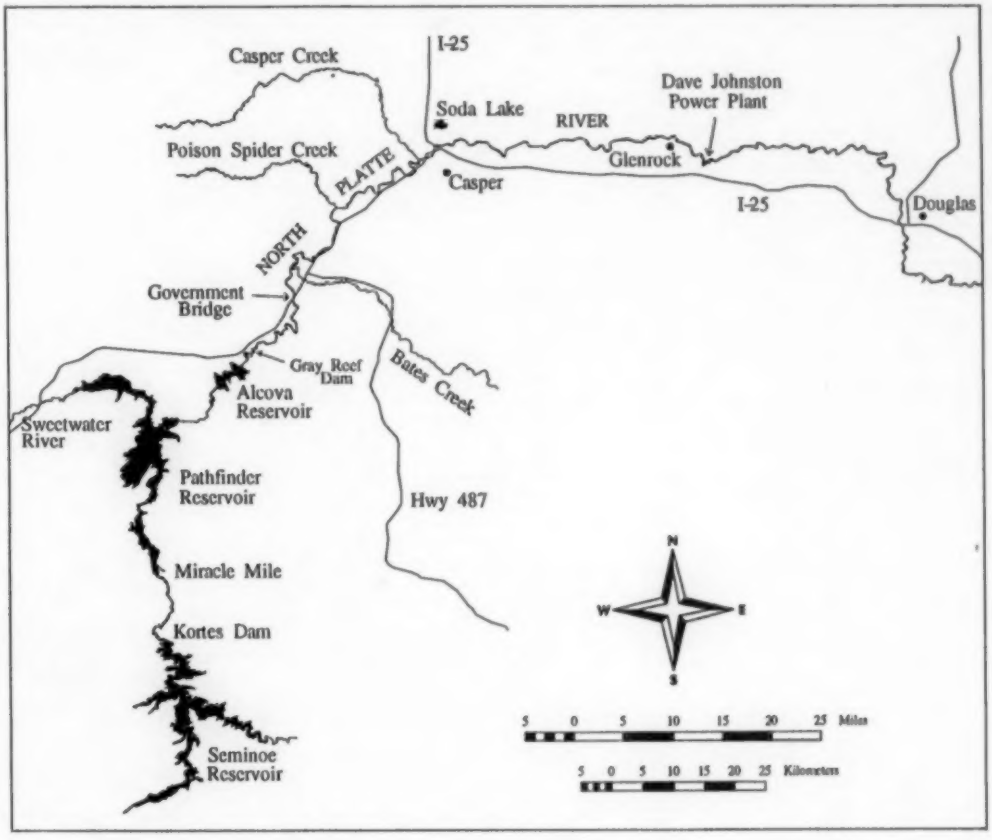
Fig. 1. Study area including the North Platte River, Soda Lake, and reservoirs in Wyoming that were aerially surveyed for double-crested cormorants and American white pelicans in 1993 and 1994.



In cases where cormorants or pelicans eat sport or commercial fish, are amounts eaten cause for concern? A number of studies have combined food habits data with estimates of bird energy requirements to calculate amounts of different fish species consumed (Furness 1978; Furness and Cooper 1982; Wiens 1984; Guillet and Furness 1985; Birt et al. 1987; Barrett et al. 1990; Linn and Campbell 1992; Glahn and Brugger 1995; Madenjian and Gabrey 1995). Results suggest that piscivorous birds can substantially affect fish stocks, depending on bird densities and availability of alternative prey. There have been no energetics studies on cormorants or pelicans in cold-water rivers. Also, few energetics analyses have considered the chronology of amounts, species, and sizes of fish consumed by breeding and nonbreeding birds and their young. These aspects are critical to bird impacts on prey population processes (Gutreuter and Anderson 1985), and to design of stocking strategies to minimize bird predation.

We wished to estimate the magnitude of double-crested cormorant and American white pelican predation on fingerling rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout stocked into the North Platte River, Wyoming, as well as on wild trout, suckers (*Catastomus* spp.), and minnows (Cyprinidae). We determined bird diets by collecting adult cormorants and pelicans from the river. To estimate numbers and biomass of fish consumed, we combined diet information with population estimates of adult cormorants, chick cormorants, and adult pelicans in a computer simulation model of bird energy demand. We also performed uncertainty analyses (e.g., Lovvorn and Gillingham 1996) to determine which parameters explained the most variation in model estimates of the amounts of fish eaten.

Methods

Our study was conducted on the North Platte River upstream of Casper, Wyo., to Gray Reef Dam, and on a double-crested cormorant colony at Soda Lake, 2.5 km north of Casper (Fig. 1). Cormorants were nesting on two islands in Soda Lake, but there were no known breeding colonies of pelicans within 110 km of our study area. Water depth and speed in this part of the North Platte River vary widely among riffles, channels, and pools mostly <3 m deep, and the location and nature of these habitats change with seasonal releases of water from dams upsteam. The 83-km section of river between Casper and Gray Reef Dam is stocked annually with fingerling trout. On 2 July 1993, this section was stocked with 195 240 rainbow trout 6–10 cm long and 27 330 rainbow trout 16 cm long. On 27 June 1994, 54 100 rainbow trout and 38 900 cutthroat trout 11–13 cm long were stocked.

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Bird population sizes

To determine numbers of cormorants and pelicans in the study area, we did aerial surveys from a Bellanca Scout aircraft at an altitude of 10–25 m and speed of 100–110 km·h^{-1}. In 1993, we flew five surveys (29 May, 24 June, 9 July, 30 July, and 16 September) of the North Platte River from Dave Johnston Power Plant to 1 km below Kortes Dam, including Soda Lake, Alcova Reservoir, Pathfinder Reservoir, and 5 km of the Sweetwater River (Fig. 1). In 1994, we flew seven surveys of the same area (16 April, 31 May, 23 June, 7 July, 20 July, 16 August, and 18 September). All surveys started between 06:15 and 08:00 and lasted about 3 h.

Numbers of cormorant chicks were estimated from ground surveys of the colony on 15 May, 31 May, 11 June, and 24 June 1994. Surveys were too infrequent to yield accurate hatching and mortality rates and were done only to estimate chick numbers. During each survey the total number of active nests was counted on both islands. Total number of chicks was estimated by multiplying the number of active nests by the mean number of chicks per nest. Mean number of chicks per nest was determined from 150 nests on the east island that were individually marked with numbered stakes before cormorants arrived. All surveys of nests and young were done with flashlights between 02:30 and 04:00 to minimize predation on cormorant eggs and chicks by California gulls (*Larus californicus*) nesting in the colony.

Bird diets

Esophagus and gizzard contents were determined for cormorants and pelicans collected with shotguns on the North Platte River between Casper and Gray Reef Dam. Birds were collected throughout daylight hours. In 1993, cormorants (no pelicans) were collected during four sampling periods: sample period 1 (10–30 June, $n = 10$) was before trout stocking on 2 July, and sample periods 2 (5–16 July, $n = 11$), 3 (19–30 July, $n = 10$), and 4 (23–27 August, $n = 6$) were after stocking. In 1994, cormorants were collected during five sampling periods and pelicans during three sampling periods: sample periods 1 (12–18 May, $n = 10$) and 2 (2–23 June, $n = 12$) for cormorants were conducted before stocking on 27 June, and sample periods 3 (30 June – 14 July, $n = 11$), 4 (18–28 July, $n = 10$), and 5 (10–18 August, $n = 9$) were after stocking. For pelicans (collected in 1994 only), sample period 1 (2–23 June, $n = 4$) was before stocking, and sample periods 2 (30 June – 14 July, $n = 5$) and 3 (10–18 August, $n = 5$) were after stocking. In tables and figures in this paper, adult cormorants include breeding and nonbreeding adults, and subadults hatched in previous years; pelicans include nonbreeding adults and subadults. (Cormorants and pelicans do not breed until 2–3 years old.)

From cormorants and pelicans collected on the river, we removed the esophagus and gizzard as soon as birds were retrieved and stored them in a cooler for 1–4 h before processing. In the laboratory, food contents were removed, whole fish were identified and measured, and otoliths were retained for analysis (Derby and Lovvorn 1997). Otoliths were later mounted in epoxy on microscope slides, and sanded (without prior sectioning) with 600-grit sandpaper to expose growth rings (Thompson and Beckman 1995). Mounted otoliths were examined at 40× magnification under a compound microscope, and the maximum distance between the center of the nucleus (Williams and Bedford 1974) and the edge of the thick end of each otolith was measured with an ocular micrometer. This method was intended to reduce error in measurements of total otolith length caused by variable erosion of the opposite, thinner end of the otolith. We also excluded from analysis otoliths that were clearly eroded (Suter and Morel 1996). Estimates of fish length were based on separate regression equations relating total fish length (L; mm) to otolith radius (X; μm) for trout ($R^2 = 0.90$, $n = 20$), suckers (*Catastomus commersoni* and *C. catastomus*) ($R^2 = 0.94$, $n = 42$), and minnows (mainly *Rhinichthys cataractae* and *Pimephales promelas*) ($R^2 = 0.92$, $n = 18$):

$$(1) \quad L_t = 78.1 - 0.056X + 9.0 \times 10^{-5} X^2$$

$$(2) \quad L_s = -54.9 + 0.207X$$

$$(3) \quad L_m = -30.0 + 0.183X$$

where L_t, L_s, and L_m are total length for trout, suckers, and minnows, respectively. We matched pairs of otoliths (two occur in each fish) whenever possible, so numbers of fish reported equal the number of matched pairs plus the number of unmatched otoliths.

Bioenergetics model

We used a bioenergetics model, written in QuickBasic 4.5, which incorporated population estimates, food habits, and energy requirements to estimate the total number and biomass of fish consumed according to fish type (trout, sucker, or minnow) and length-class. Energy requirements of the cormorant and pelican populations were calculated by multiplying the estimated energy requirement of a single chick cormorant, adult cormorant, or pelican by the estimated population size of chick cormorants, adult cormorants, and pelicans, respectively.

For adult cormorants and pelicans, we used eqs. 5.35 and 5.28 (converted from kcal to kJ) of Kendeigh et al. (1977) to estimate daily existence energy (EE; kJ·day^{-1}) from body mass (M; g) at air temperatures of 0 and 30°C:

$$(4) \quad EE = 17.34M^{0.5444}, \text{ at } 0°C$$

$$(5) \quad EE = 4.472M^{0.6637}, \text{ at } 30°C$$

For M, we used 2000 g for cormorants and 6500 g for pelicans. Existence energy of adults was interpolated for mean temperature on a given day from values calculated from the above equations. Existence energy is the energy required by captive birds without costs of flight, food searching, predator avoidance, etc., incurred by free-ranging birds. In the absence of time–activity budgets for wild cormorants and pelicans in our study, the fraction of EE added to account for activity energy (AE) was assumed to be 40%, where total daily energy requirement equals EE + AE (see Furness 1978).

These equations yield values comparable with those derived by other means. Based on analyses of otoliths in pellets (indigestible residue) regurgitated daily by individual great cormorants (*Phalacrocorax carbo*) at their nests, Platteuw and Van Eerden (1995) estimated a mean daily energy intake of 0.467 kJ·g^{-1} for birds assumed to weigh 2600 g. Based on detailed time-activity budgets and measurements of the costs of different activities, Gremillet et al. (1995) estimated daily energy costs (kJ·g^{-1}) for great cormorants assumed to weigh 2230 g of 0.347 for adults laying and incubating eggs, 0.419 for adults rearing small chicks, and 0.475 for adults rearing large chicks. Based on time–activity budgets, multiples of basal metabolic rate for each activity, and estimates of thermoregulation costs, double-crested cormorants wintering in Mississippi with mean body mass of 2098 g and mean daily temperature of 11.4°C had a daily energy cost of 0.820 kJ·g^{-1} (Glahn et al. 1995). For the same temperature and body mass, eqs. 4 and 5 above, plus 40% AE, yield a daily energy cost of 0.643 kJ·g^{-1}.

To our knowledge, the only published measurements of energy costs in grown pelicans are for a captive great white pelican (*Pelecanus onocrotalus*) allowed only to sit and stand, whose fish consumption in July indicated a daily EE of 0.353 kJ·g^{-1} (Cooper 1980). At a summer temperature of 20°C, eqs. 4 and 5 above yield a daily EE of 0.232 kJ·g^{-1} for an 8900-g great white pelican and 0.262 kJ·g^{-1} for a 6500-g American white pelican. Thus, eqs. 4 and 5 might underestimate EE for pelicans, but we had few data by which to adjust the values. Moreover, the soaring flight and surface feeding of pelicans is probably less costly than the flapping flight and pursuit diving of cormorants. In keeping with Guillet and Furness' (1985) use of eqs. 4 and 5 in their model for great white pelicans, we continued to use these equations and an activity energy increment of 40% of EE for our model of American white pelicans.

Calculating the energy needs of chick cormorants required estimates of body mass of growing chicks on a daily basis. Furness and

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Table 1. Parameter values used for estimating fish consumed by double-crested cormorants and American white pelicans on the North Platte River, and ranges of those parameters used in uncertainty analyses of the model.

Parameter	Value	Range
Fraction of EE used for AE	0.40	0.36–1.13
Digestive efficiency (%)	80	70–90
Energy content of fish (kJ·g^{-1} fresh mass)	Trout: 7.3 Suckers: 3.0 Minnows: 5.8	All species: 3–8
No. of adult cormorants	From aerial counts	±20%
Adult cormorant EE	From eqs. 4 and 5	±10%
No. of pelicans	From aerial counts	±20%
Pelican EE	From eqs. 4 and 5	±10%
No. of cormorant chicks	From nest counts	±10%
Body mass of cormorant chicks	From eq. 7	±10%
Energy requirement of cormorant chicks	From eq. 8	±10%
Daily survival of cormorant chicks (%)	99	98–99.9[a]

Note: Adult cormorants include breeding and nonbreeding adults, and subadults hatched in previous years. Pelicans include nonbreeding adults and subadults. EE, existence energy; AE, activity energy.

[a]Corresponds to fledging rates of 40–95% in a 45-day prefledging period.

Cooper (1982) estimated the daily M (g) of cape cormorant (*Phalacrocorax capensis*) chicks to be

$$M = 1500\exp(-4.0558e^{-0.0795N}) \quad (6)$$

where 1500 g is the asymptotic chick mass and N is chick age in days. Asymptotic body mass for double-crested cormorant chicks was reported as 1350 g for captive-reared birds in Florida (Cummings 1987) and, for wild birds, 1690 g in Quebec (DesGranges 1982), 1900 g in New Hampshire (Dunn 1975*b*, 1976), and 2440 g in British Columbia (Robertson 1971). We used the equation:

$$M = 2000\exp(-4e^{-0.09N}) \quad (7)$$

which agrees closely with the growth data of Dunn (1975*b*). We then estimated daily energy requirements (E; kJ·day^{-1}) from eq. 5.78 in Kendeigh et al. (1977) (converted from kcal to kJ):

$$E = 5.665M^{0.814} \quad (8)$$

where M is calculated from eq. 7 above. Equation 8 yields values generally two to three times higher than data for double-crested cormorant chicks resting in a respirometry chamber with no wind (Dunn 1976). These higher calculated values are appropriate, given the added energy cost of convective heat loss on islands without windbreaks at Soda Lake, where mean daily wind speed during the nestling period was 18 km·h^{-1} (cf. Goldstein 1983; Chappell et al. 1989).

We converted total energy required to total mass of fish consumed using estimates of the energy content of fish and digestive efficiency. Energy content (kJ·g^{-1} fresh mass) was taken to be 7.3 for trout, 3.0 for suckers, and 5.8 for minnows, based on data and equations in Lalancette (1976), Cui and Wootton (1988), Bennett and Hart (1993), and Hartman and Brandt (1995). Digestibility of fish was assumed to be 80%, based on measurements in captive double-crested cormorants (Dunn 1975*a*; Cummings 1987; Brugger 1993) and a great white pelican (Cooper 1980). The mass of fish consumed from the North Platte River between Casper and Gray Reef Dam was estimated by multiplying the total mass of fish consumed by adult cormorants and pelicans by the fraction of adult cormorants and pelicans counted in that section of river. This same fraction of adult cormorant use was applied to the chick cormorant population to estimate the mass of fish consumed from this section of river by chick cormorants. Food habits data were used to partition the total mass of fish required into masses of trout, suckers, and minnows in each length class. Allometric relations of fresh mass (M; g) versus length (L; mm) for fish collected from the North Platte River were used to calculate the average mass of fish in a given length-class, which was then used to calculate the number of fish consumed in each length-class. These allometric equations were $M = 1.064 \times 10^{-5} L^{3.006}$ ($R^2 = 0.99$, $P < 0.01$, $n = 25$) for trout; $M = 4.746 \times 10^{-6} L^{3.170}$ ($R^2 = 0.99$, $P < 0.01$, $n = 53$) for suckers; and $M = 1.240 \times 10^{-5} L^{2.920}$ ($R^2 = 0.92$, $P < 0.01$, $n = 11$) for minnows. We captured few or no fish from the 0–8 cm length-class, so constant masses were used for this length-class of trout (2 g), suckers (2 g), and minnows (1.5 g).

Uncertainty analyses

We did uncertainty analyses of the simulation model (cf. Lovvorn and Gillingham 1996) to evaluate effects of variation in selected parameters on model estimates of fish consumed by cormorants and pelicans. Such analyses focus future research on variables that need accurate measurement for reliable model estimates and that have the greatest influence on bird-fishery interactions. In our analysis, we varied parameters within ranges reported for cormorants or other seabirds, or within set percentages (Table 1). Because of large variation in the parameters, we were concerned with sampling between endpoints of the ranges and not from particular probability distributions. Thus, all parameters were considered to be uniformly distributed within specified ranges. The magnitude of ranges used has large effects on the sensitivity of model estimates to variation in different parameters. Consequently, we varied each parameter between endpoints likely to occur in nature, rather than between constant percentages that were unrealistically large for some parameters and too small for others.

Energy costs of activity were calculated by multiplying EE (from eqs. 4 and 5) by a fraction ranging from 0.36 to 1.13. Furness (1978) estimated extremes of activity costs to be 36% of EE for great skuas (*Catharacta skua*) and 113% for Arctic terns (*Sterna paradisaea*). Energy content of all fish was varied from 3 to 8% (Lalancette 1976; Cui and Wootton 1988; Bennett and Hart 1993; Hartman and Brandt 1995) and digestibility of fish from 70 to 90% (Dunn 1975*a*; Cummings 1987; Brugger 1993). Fledging rates (number of chicks hatched that fledged) of double-crested cormorants averaged 95% (Drent et al. 1964), 96% (van Tets 1959), and 95.6% (Robertson 1971) on an island in coastal British Columbia, but only 80% for pelagic cormorants (*Phalacrocorax pelagicus*) on the same island (van Tets 1959). For uncertainty analyses, we varied daily chick survival from 98 to 99.9%, which corresponds to approximate fledging rates of 40 to 95% for a 45-day prefledging period.

To assess effects of daily fluctuations in numbers of birds feeding

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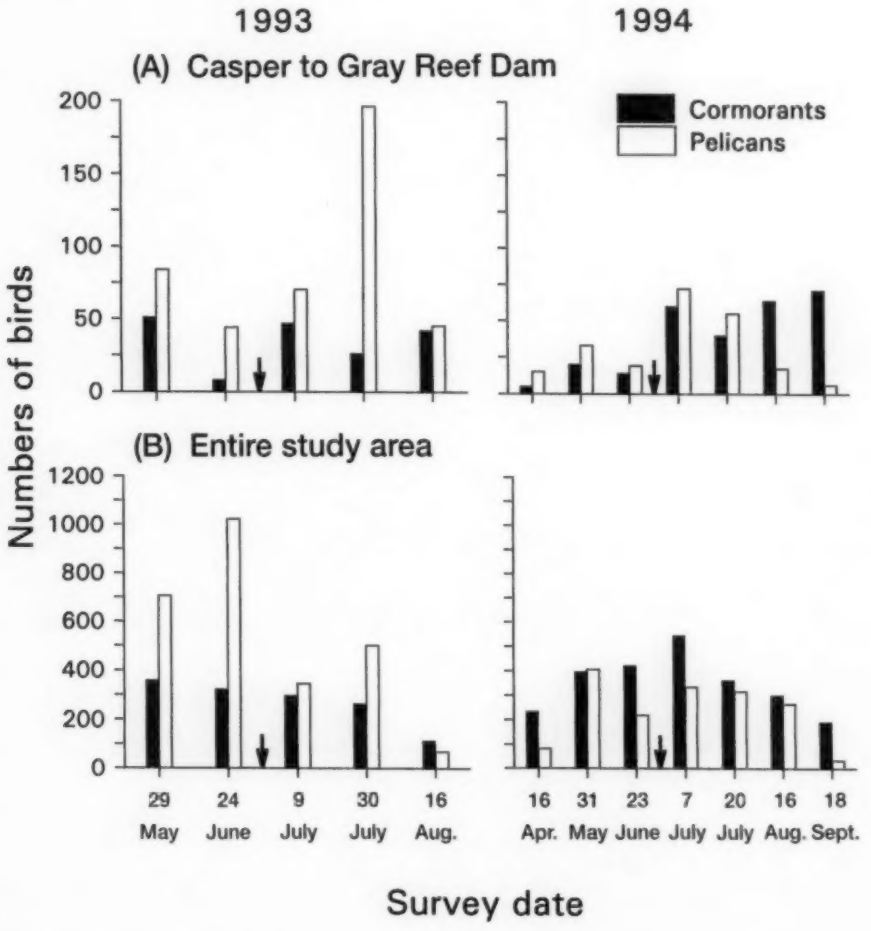
Fig. 2. Counts of double-crested cormorants and American white pelicans on the North Platte River between the city of Casper and Gray Reef Dam, Wyoming, and in the entire study area including reservoirs and Soda Lake (Fig. 1), during aerial surveys in 1993 and 1994. Surveys started at the Dave Johnston Power Plant and ended 1 km below Kortes Dam. Fingerling trout were stocked on 2 July 1993 and 27 June 1994 (shown by arrows).



in the river, numbers of adult cormorants and pelicans were varied by ±20% from daily estimates that were linearly interpolated from periodic aerial surveys of the section from Casper to Gray Reef Dam (Fig. 2). Number of chick cormorants, chick mass and energy requirements, and EE of pelicans and adult cormorants were varied by ±10%.

The uncertainty analysis entailed 150 simulations in a Latin hypercube design (Swartzman and Kaluzny 1987). The uniform distributions of each parameter were divided into 150 equal intervals and, for each simulation, values for each parameter were randomly selected from intervals chosen randomly without replacement. Multiple regression was performed with number of fish as the dependent variable and the 11 varied parameters (Table 1) as the independent variables. Relative partial sums of squares (RPSS) were used to compare the relative effects of individual parameters on the total number of fish consumed, with effects of all other parameters statistically removed. RPSS values thus indicate the unique contribution of each independent variable to total variation, while the other independent variables are also fluctuating. This approach indicates, within constraints of the model, which parameters need more investigation to improve estimates of the total number and biomass of fish consumed by cormorants and pelicans.

Results

Bird population sizes

Counts of cormorants and pelicans over the entire study area (the river, reservoirs, and nesting islands, excluding chicks), as well as on the river between Casper and Gray Reef Dam only, varied among survey dates in both 1993 and 1994 (Fig. 2). Omitting counts on 16 April and 18 September 1994 to allow comparison between similar survey dates, numbers of cormorants increased from 1993 to 1994 in the entire study area (paired t test, $P = 0.02$) but not from Casper to Gray Reef Dam ($P = 0.65$). Counts of cormorants in the entire study area were 272 ± 94 (mean ± SD) in 1993 and 365 ± 119 in 1994. For pelicans, counts from Casper to Gray Reef Dam decreased from 1993 to 1994 ($P < 0.01$) but, because of high variances (Fig. 2), did not differ significantly between years in the entire study area ($P = 0.36$). Total counts of pelicans in the entire area were 529 ± 361 in 1993 and 294 ± 128 in 1994. The percentage of cormorants and pelicans in the section of river between

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Table 2. Percentage of double-crested cormorants and American white pelicans counted in the entire study section of the North Platte River (excluding nesting islands in Soda Lake) that were counted in the section between Casper, Wyo., and Gray Reef Dam in 1993 and 1994 (see Fig. 1).

	Cormorants		Pelicans	
Year	Before stocking	After stocking	Before stocking	After stocking
1993	2.5	15.8*	4.3	20.3*
1994	3.3	11.0*	8.6	21.4*

*Significantly different from percentage before stocking (percentage test, $P < 0.03$; Sokal and Rohlf 1969, pp. 607–608).

Casper and Gray Reef Dam increased after that section was stocked in both years (Table 2). In the bioenergetics model, we used counts from Casper to Gray Reef Dam in 1994 when data on numbers of chicks at Soda Lake were also collected.

Numbers of cormorant chicks in nests marked before cormorants arrived in 1994 were zero ($n = 56$ nests) on 15 May, 2.65 ± 1.13 ($n = 79$) on 31 May, 2.33 ± 0.71 ($n = 88$) on 11 June, and 1.01 ± 1.11 ($n = 119$) on 24 June. Although no chicks were observed in marked nests during the first survey, there were a small number of very young chicks in unmarked nests. By multiplying these values by the total number of active nests counted on both islands in Soda Lake, we estimated the total number of chicks to be zero on 15 May, 822 on 31 May, 697 on 11 June, and 205 on 24 June. The large decline in numbers between the third and fourth surveys resulted from many chicks growing large enough to leave their nests when disturbed by our presence. Surveys were too infrequent to yield accurate hatching and mortality rates and were done only to estimate chick numbers.

In the simulation model, we used a total of 820 chicks hatching over a 20-day period. We arbitrarily specified that 660 chicks (80%) hatched at a rate of 55 day^{-1} in the first 12 days, and the remaining 160 chicks (20%) hatched at a rate of 20 day^{-1} in the last 8 days to represent late-nesting and renesting birds. Simulated growth and energy demands of the cohort of chicks hatched on a given day were calculated separately for each cohort on subsequent days. Chick numbers were reduced on a daily basis according to daily chick survival (Table 1) until fledging.

Bird diets

In collected cormorants, the percentage of fish numbers in each length-class did not differ between 1993 and 1994 (analysis of variance on ranked data, $P = 0.64$), so we averaged these data across years to increase sample size for results used in the model. In terms of fish numbers, trout were 29 and 5% of the cormorant diet in sampling periods before stocking versus 52, 41, and 86% in sampling periods after stocking (Fig. 3). For pelicans (sampled in 1994 only), trout were only 1% of the diet before stocking and 34 and 9% in sampling periods after stocking (Fig. 3).

In terms of biomass (fresh mass; Fig. 4), the proportion of trout, suckers, and minnows in each length-class found in cormorants did not differ between 1993 and 1994 (analysis of variance on ranked data, $P = 0.32$), so these data were averaged between years. In sampling periods before stocking, suckers were 85 and 84% of cormorant prey biomass, but trout were 77, 71, and 98% in sampling periods after stocking. For pelicans, suckers were 84, 92, and 77% of prey biomass during sampling periods in 1994 (Fig. 4) (no pelicans were collected in 1993).

Fragments of crayfish (Cambaridae) and tiger salamanders (*Ambystoma tigrinum*) were present in 17 and 25%, respectively, of collected cormorants before stocking and in 9 and 0% after stocking. These fragments could not be quantified as fractions of prey numbers or biomass (Derby and Lovvorn 1997). Thus, model estimates of bird consumption of fish (see below) are inflated by the amount of dietary energy provided by crayfish and salamanders.

Bird energy requirements

Energy requirements of adult cormorants, chick cormorants, and pelicans obtaining their food from the section of river from Casper to Gray Reef Dam (based on the feeding distributions of adults) were estimated with constant parameters in the energetics model (Table 1). Peak energy demands of adult and chick cormorants, as well as total peak demand, occurred shortly after trout stocking on 27 June (Fig. 5).

We also used the bioenergetics model, with fixed parameters (Table 1), to estimate consumption of trout, suckers, and minnows in each length-class between Casper and Gray Reef Dam in 1994 (Fig. 6). Overall, about equal total numbers of trout, suckers, and minnows were consumed, but most trout and minnows 0–16 cm long were eaten by cormorants, whereas suckers 0–16 cm long were eaten by both cormorants and pelicans. Much larger numbers of trout were consumed (mainly by cormorants) in the 8–16 cm length-class (an estimated 78 000 trout) than in any other length-class of trout. A large fraction of stocked trout are in this 8–16 cm length-class.

We then estimated total biomass (fresh mass) of trout, suckers, and minnows consumed on a daily basis from Casper to Gray Reef Dam with fixed parameters (Table 1) in the model. Although cormorants and pelicans consumed about equal numbers of trout, suckers, and minnows (Fig. 6), trout and suckers contributed far more to total biomass consumed than did minnows (Fig. 7). Suckers were the main food in early spring for both cormorants and pelicans, and throughout the sampling period for pelicans. Pelicans consumed very little biomass of trout at any time of the study period, whereas trout were the major prey of cormorants after stocking (Fig. 7).

Uncertainty analyses

Uncertainty analyses were performed to determine which of 11 parameters (Table 1) explained significant variation in model estimates of the numbers of trout, suckers, and minnows consumed. RPSS were used to evaluate the fraction of total variance in the dependent variables (numbers of trout, suckers, and minnows) that was explained by each independent variable (parameter value). Energy content of fish, daily survival of chick cormorants, fraction of EE used for activity costs (AE), and digestive efficiency explained the most variation in estimated numbers of trout, suckers, or minnows consumed (Table 3). Chick body mass and chick energy requirements were the only parameters not significant in estimating the numbers of any fish type consumed. The other five parameters were of varying importance depending on fish type.

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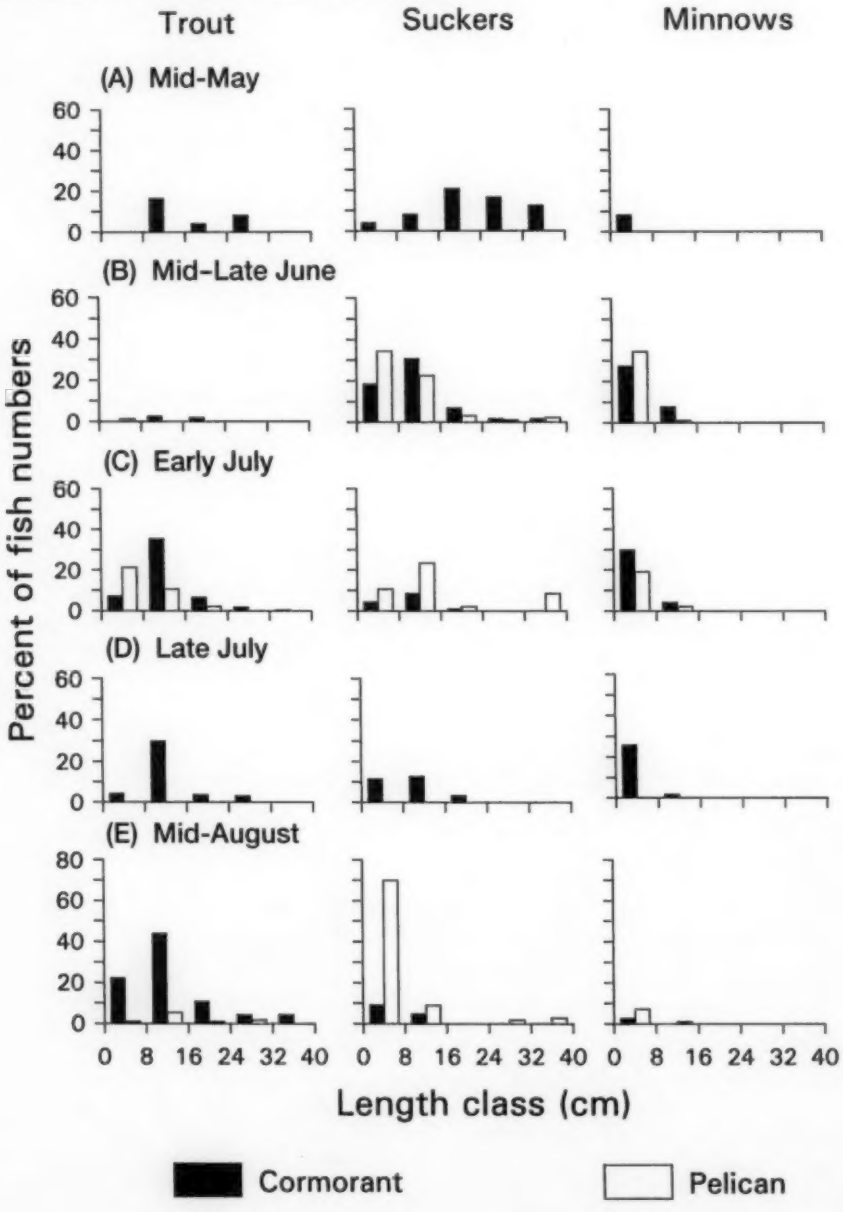
Fig. 3. Percentages of fish numbers in esophagi and gizzards of 89 double-crested cormorants and 14 American white pelicans collected on the North Platte River, Wyoming. Length-classes include the 8-cm interval below each length shown on the abscissa. Pelicans were not sampled in mid-May or late July. Fingerling trout were stocked on 2 July 1993 and 27 June 1994 (before panel C). Total numbers of fish on which percentages are based were 21 for cormorants only in mid-May; 56 for cormorants and 93 for pelicans in middle to late June, 171 and 47 in early July; 65 for cormorants only in late July; and 31 and 113 in mid-August.



Discussion

Both cormorants and pelicans altered their feeding distributions in response to trout stocking. Corresponding to greater use of stocked areas, cormorants shifted from eating mainly suckers to mainly trout, and pelicans included more trout in their diet although continuing to eat mostly suckers. Stocking dates coincided with peak food demands of piscivorous birds, which depended mainly on the chronology of food requirements of cormorant chicks. Pelicans and especially cormorants can consume a large fraction or almost all of fingerling trout stocked in the study area. However, low consumption of trout in the spring before stocking suggests that overwinter survival limited trout recruitment, regardless of bird predation (see below).

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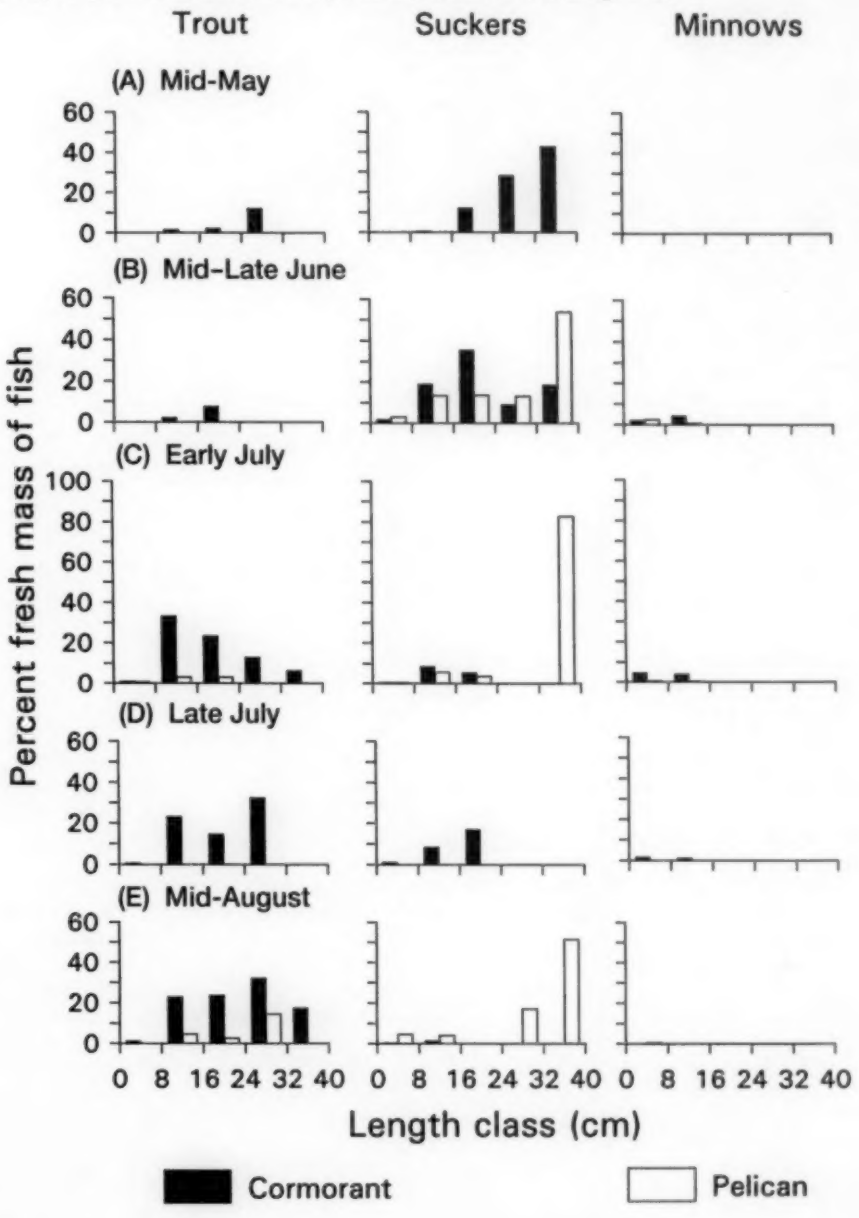
Fig. 4. Percent fresh mass of fish in esophagi and gizzards of 89 double-crested cormorants and 14 American white pelicans collected on the North Platte River, Wyoming. Length-classes include the 8-cm interval below each length shown on the abscissa. Pelicans were not sampled in mid-May or late July. Fingerling trout were stocked on 2 July 1993 and 27 June 1994 (before panel C).



Bird response to trout stocking

Piscivorous birds often feed in areas of high fish density (Eriksson 1985; Safina and Burger 1985) and are attracted to other birds in feeding areas (Wood 1985; Anderson 1991). In both years of our study, cormorants and pelicans substantially increased their use of the North Platte River between Casper and Gray Reef Dam after this section was stocked with fingerling trout (Table 2). In 1993, cormorant and especially pelican use of the stocked section of river was variable after stocking (Fig. 2). In 1994, cormorants continued to use this section of river more after stocking than before stocking, whereas pelican numbers increased initially and then declined through summer.

The fraction of sport or commercial fish in the diets of cormorants and pelicans varies greatly, from 0.6% (Hobson et al. 1989) to 93% (Modde et al. 1996), and appears to depend largely on local food availability (Whitfield and Blaber 1979; Craven and Lev 1987; Draulans 1988; Trayler et al. 1989). Therefore, birds feeding in heavily stocked areas are expected

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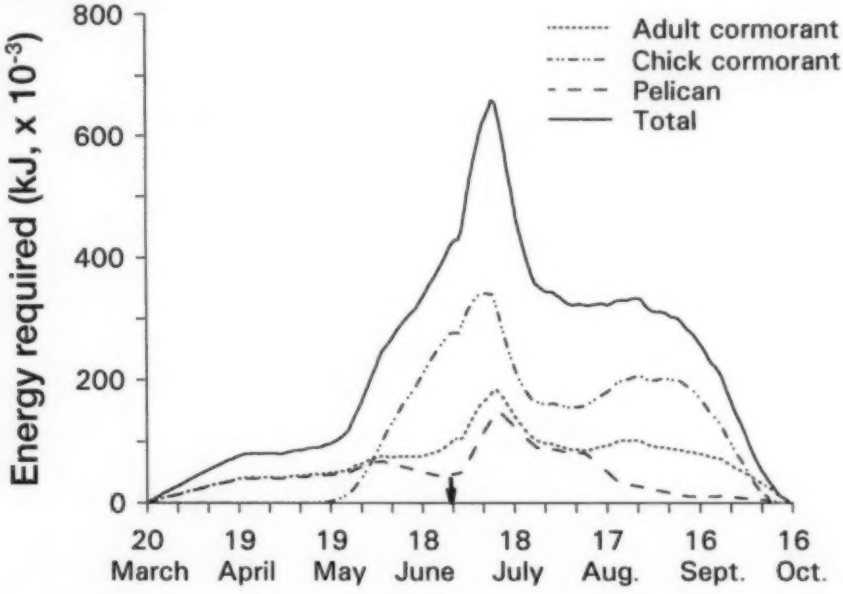
Fig. 5. Estimated energy requirements of double-crested cormorant and American white pelican populations at the Soda Lake cormorant colony and on the North Platte River from the city of Casper to Gray Reef Dam, Wyoming, in 1994. Adult cormorants include breeding and nonbreeding adults and subadults hatched in previous years. Pelicans include nonbreeding adults and subadults. Fingerling trout were stocked on 27 June 1994 (shown by arrow).



to have much of their diet comprised of the stocked species (Draulans 1988). In contrast to cormorants, American white pelicans generally eat only small proportions of economically valuable fish (Lingle and Sloan 1980; Anderson 1987; Findholt and Anderson 1995), except for cutthroat trout in one report (Davenport 1974).

Our findings are consistent with the above results. Before trout stocking, suckers were the main prey of both cormorants (61% of numbers, 84% of fresh mass) and pelicans (64% of numbers, 85% of mass), while few trout were eaten by either cormorants (17% of numbers, 12% of mass) or pelicans (1% of numbers, 0.1% of mass). However, after stocking the diet of cormorants changed to mainly trout (60% of numbers, 82% of mass) and few suckers (19% of numbers, 14% of mass), whereas pelicans continued to eat mainly suckers and minnows (79% of numbers, 86% of mass). Pelicans were not sampled in late July; but in mid-August they consumed many suckers in the 0–8 cm length-class (Fig. 3) and substantial biomass of suckers in greater length-classes (Fig. 4). The diet of cormorants had shown very similar patterns earlier in the study period. Thus, the shift in the cormorant diet appeared to result from trout stocking and not from decreased availability of suckers. Higher vulnerability of stocked trout (Fraser 1974; Berejikian 1995) might contribute to increased exploitation of these fish.

Most published studies of cormorant and pelican predation report only means or ranges for the mass or length of fish consumed (e.g., Hobson et al. 1989; Trayler et al. 1989; Anderson 1991; Ottenbacher et al. 1994). Double-crested cormorants ate mostly fish 12–15 cm long in Lake Superior (Craven and Lev 1987), 3–10 cm long in Texas reservoirs (Campo et al. 1993), and 10–20 cm long in an area of catfish farms in Mississippi (Glahn et al. 1995). In terms of fish numbers, both cormorants and pelicans in our study ate mainly trout of small (stocked) length classes (<16 cm), although both cormorants and pelicans did eat trout and suckers that were much larger (Figs. 3 and 4). This pattern might indicate that cormorants and pelicans in our study were selecting smaller trout (0–16 cm) or that smaller (mainly stocked) trout were more available.

Uncertainty analyses

Model estimates of the impact of bird predation on fish are only as reliable as model algorithms and the data they use. Our uncertainty analyses revealed several parameters whose accuracy strongly affects our model predictions (Table 3).

Energy content of fish was the most important factor in estimating numbers of fish consumed by cormorants and pelicans. Consequently, we recommend use of species-specific energy densities of prey in energetics modeling (see Hartman and Brandt 1995). The fraction of EE used for the cost of activity (AE) was also an influential parameter (Table 3). Unlike Furness (1978), who found EE to be very important in Monte Carlo simulations, we found that AE was consistently more influential than cormorant *EE*, pelican *EE*, or energy required by chicks. Determining accurate activity costs is difficult, and estimates used by different authors are so disparate that results might differ appreciably depending on values used. Different estimators include (*i*) time–activity budgets with estimates of the cost of each activity (Furness 1978; Furness and Cooper 1982; Weathers et al. 1984; Guillet and Furness 1985; Gremillet et al. 1995); (*ii*) consumption rate in some proportion to body mass, often determined in captive birds (Linn and Campbell 1992; Ottenbacher et al. 1994); (*iii*) multiples of

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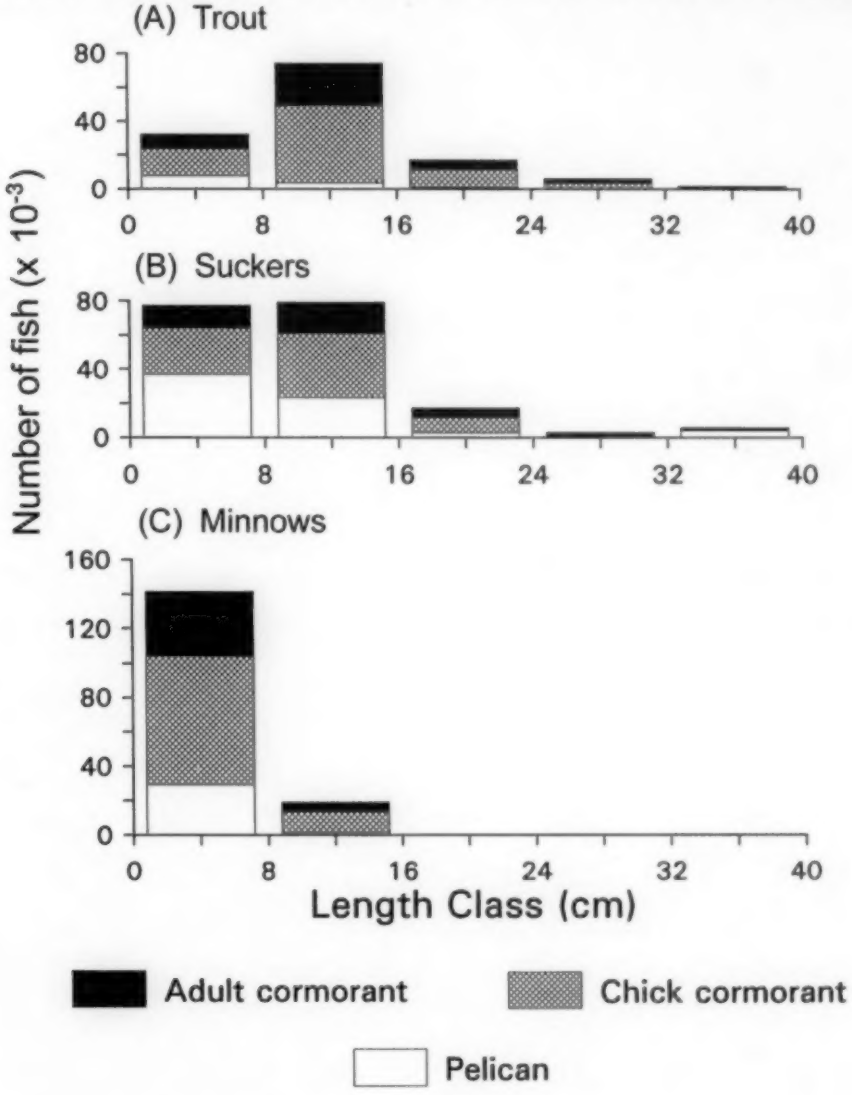
Fig. 6. Estimated total numbers of fish consumed by double-crested cormorants (including chicks) and American white pelicans feeding in the North Platte River from the city of Casper to Gray Reef Dam, Wyoming (Fig. 1), in 1994. Species and age conventions are as in Fig. 5.



basal metabolic rate (Barrett et al. 1990); and (*iv*) doubly labelled water measurements (Weathers et al. 1984; Birt-Friesen et al. 1989). For cormorants, estimates from equations based on doubly labelled water measurements (see Madenjian and Gabrey 1995) are 1.4–4 times higher than values derived from the other approaches, which are generally similar (see Methods). Ideally, several of these methods should be compared for a given situation, and model results should always be viewed in light of their strong dependence on activity cost estimates.

Digestive efficiency, which we varied from 70-90%, was also an important variable in our model (Table 3). There were no data available on digestibility of fish by pelicans, and data for cormorants were from captive rather than wild birds. Because digestibility can vary among fish species (Brugger 1993), it is desirable to measure digestibilities for trout, suckers, and minnows in both cormorants and pelicans.

The other significant parameters were daily chick survival, adult cormorant numbers, chick cormorant numbers, and pelican numbers. Daily chick survival and chick numbers could be measured more accurately by detailed study of marked nests, and cormorant and pelican populations could be better determined with more frequent and possibly more extensive aerial surveys.

Impacts on fish: bird consumption versus overwinter survival

Several criteria have been used to evaluate whether piscivorous birds impact populations of valuable fish species. Early

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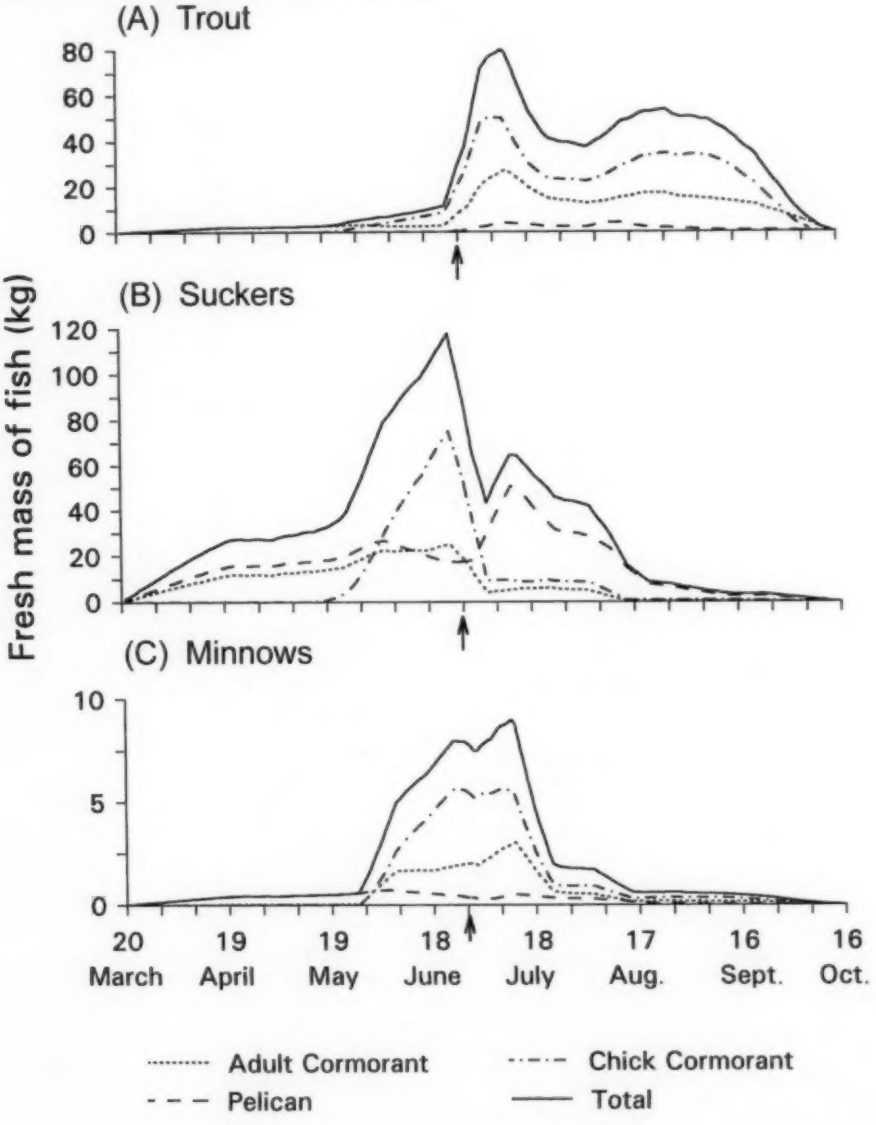
Fig. 7. Estimated fresh mass of trout, suckers, and minnows consumed by double-crested cormorants (including chicks) and American white pelicans on the North Platte River from the city of Casper to Gray Reef Dam, Wyoming (Fig. 1), in 1994. Species and age conventions are as in Fig. 5. Fingerling trout were stocked on 27 June 1994 (shown by arrows).



studies presumed that, if bird diets contained 5% or more valuable fish, the birds were impacting the populations (Draulans 1988). To evaluate effects of bird predation, it is best to compare estimated amounts of fish consumed with total populations or production of fish (Draulans 1988; Wiens 1984; Madenjian and Gabrey 1995). The Wyoming Game and Fish Department has estimated trout populations in the North Platte River between Casper and Gray Reef Dam but only for trout ≥15 cm long. Thus, their estimates do not cover our first length-class (0–8 cm) or most of our second length class (8–16 cm). Our attempts to assess trout, sucker, and minnow populations in these lower length-classes by boat-based electroshocking were not satisfactory, because we were not confident that the scarcity of small trout in samples before stocking reflected scarcity in the river.

Nevertheless, few trout in the 0–8 and 8–16 cm length-classes were consumed by the birds before stocking, whereas large numbers were eaten after stocking (Figs. 3 and 4). Thus, it appears that few fish of these lengths were present after winter and that most fish of these lengths eaten in later periods came from stocking. Birds collected in the last sampling period (mid-August) contained many trout in the 0–8 and 8–16 cm length-classes (Fig. 3), so it appears that these sizes of trout were surviving until early autumn. Because few trout were

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Table 3. Relative partial sums of squares (RPSS) and partial coefficients of determination (r^2) for numbers of fish consumed by cormorants and pelicans, based on ranked data.

	Trout		Suckers		Minnows	
Parameter	RPSS ($\times 10^{-6}$)	Partial r^2	RPSS ($\times 10^{-6}$)	Partial r^2	RPSS ($\times 10^{-6}$)	Partial r^2
Energy content of fish	371 115	0.84*	273 327	0.88*	331 548	0.87*
Daily chick survival	179 566	0.71*	38 744	0.52*	96 292	0.66*
Fraction of EE used for AE	73 115	0.50*	36 665	0.50*	44 117	0.47*
Digestive efficiency	32 701	0.31*	23 531	0.40*	29 043	0.37*
No. of adult cormorants	15 350	0.18*	8 116	0.18*	12 768	0.20*
No. of cormorant chicks	10 480	0.13*	3 121	0.08*	6 729	0.12*
Adult cormorant EE	7 396	0.09*	1 144	0.03*	3 024	0.06*
No. of pelicans	825	0.01	5 866	0.14*	1 689	0.03*
Pelican EE	194	<0.01	1 031	0.03*	248	<0.01
Chick energy requirement	96	<0.01	501	0.01	838	0.02
Body mass of cormorant chicks	66	<0.01	176	<0.01	146	<0.01

Note: Uncertainty analyses consisted of 150 simulations, with values for each parameter randomly selected (for each simulation) from ranges in Table 1 by the Latin hypercube method. RPSS values indicate the relative magnitudes of effects of individual parameters on total numbers of trout, suckers, and minnows consumed, with effects of all other parameters statistically removed. Multiple R^2 was 0.91–0.93 individually for trout, suckers, and minnows. EE, existence energy; AE, activity energy.

*$P < 0.05$.

consumed by birds before stocking, the trout that lived until autumn either were not surviving winter, were leaving the sampling area, were growing out of sizes most available to cormorants, or were developing predator avoidance characteristics.

High overwinter mortality of small trout seems plausible in years such as 1993. In that year, over 200 000 trout were stocked and probably only 100 000 were consumed by birds, but the birds nevertheless did not eat trout after the winter until more were stocked. Griffith and Smith (1995) estimated that none of the 1992 cohort of rainbow trout in the Henrys Fork of the Snake River survived the winter in their Idaho study area. Thus, even in years when numbers of trout stocked greatly exceed estimated bird predation, high overwinter mortality might still eliminate recruitment. Low survival of stocked trout in general (Schuck and Kingsbury 1948; Miller 1952), and especially low overwinter survival in cold-water streams in this region (Smith and Griffith 1994; Griffith and Smith 1995), should be considered in evaluating net effects of bird predation.

Stocked fisheries and fish-eating birds

In 1994, cormorants and pelicans consumed an estimated 74 162 trout in the 8–16 cm length-class from the North Platte River between Casper and Gray Reef Dam (Fig. 6A). Only 93 000 trout 11–13 cm long were stocked that year, so these birds potentially consumed a large fraction of trout released. Fish consumption is overestimated to an unknown extent by our not considering crayfish and salamanders, which occurred in diet samples but could not be quantified as fractions of prey numbers or biomass (Derby and Lovvorn 1997). Nevertheless, if bird predation indeed limited trout survival, our results suggest several factors that might be manipulated by managers to reduce predation losses.

In terms of stocking strategies, either timing of release or fish size at release might be altered. Typical stocking dates in this region coincide closely with peak food demands of piscivorous birds if those birds are nesting locally (Fig. 5). Timing of peak demand in our study area depended mainly on the chronology of food requirements of cormorant chicks. Stocking earlier in the season might allow naive fingerlings to develop better predator avoidance behaviors before the peak of bird consumption, but lower food availability for fingerlings released earlier might offset any decreased predation (cf. Cunjak and Power 1987). Stocking later in the season would incur greater costs of feeding fish longer in hatcheries. Because we were unable to obtain reliable estimates of prey availability, it is unclear whether the birds actually selected prey in the length-classes stocked or would have responded to high availability of stocked fish regardless of size. Both cormorants and pelicans were certainly capable of eating larger fish (Figs. 3 and 4), and we suspect these birds would readily consume larger fish if they were stocked. Thus, the expense of stocking bigger fish to avoid bird predation might not be justified, if in fact stocking larger trout is cost effective under any circumstances (Johnson et al. 1995).

The uncertainty analyses suggest other influential parameters that might be manipulated, especially daily chick survival, number of cormorant chicks, and number of adult cormorants. There are legal, ethical, and public relations problems with disrupting reproduction of protected birds, and control at a nearby colony might not reduce numbers of nonbreeding birds that feed locally but were hatched in distant areas. For example, perhaps most pelicans in our study area were nonbreeders: the closest nesting colonies were at Wheatland Reservoir No. 3, 110 km away (a new colony present but not counted in 1993, 200–250 pairs in 1994), and at Bamforth Lake 150 km away (490 pairs in 1993, 200 pairs in 1994). Reducing local numbers of nonbreeding pelicans as well as nonbreeding cormorants might be impractical even if the public approved. Moreover, Johnson and Sloan (1978) reported one-way foraging distances of up to 305 km for pelicans nesting in North Dakota.

In cold-water rivers, overwinter survival might often limit recruitment of stocked trout regardless of bird predation. Thus, further insight into the role of fish-eating birds requires concurrent studies of trout survival and winter habitat, in addition to work on energy requirements, prey selectivity, and functional

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responses of cormorants and pelicans to fish densities (e.g., Doornbos 1979; Wood and Hand 1985).

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Phylogenetic relationships among *Coregonus* species inferred from the DNA sequence of the first internal transcribed spacer (ITS1) of ribosomal DNA

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Abstract: Phylogenetic analyses based on morphometrics, data on protein electrophoresis, and mitochondrial DNA have given conflicting phylogenetic hypotheses for the subfamily Coregoninae (*Prosopium*, *Coregonus*, and *Stenodus*). To resolve these conflicts, phylogenetic analysis of 13 taxa of Coregoninae was done using a nuclear gene, the first internal transcribed spacer (ITS1) of the ribosomal DNA (rDNA). ITS1 sequences of all North American ciscoes were identical. In agreement with analyses based on other molecular data, the genus *Stenodus* was included in *Coregonus*, and there was no support for the monophyly of the ciscoes. The analysis based on rDNA also placed *C. peled* as the sister species to *C. lavaretus*, but this may represent a case of hybridization and introgression. A length polymorphism involving a repeating unit of 65–67 base pairs was found in the ITS1. All ciscoes except *C. peled* had one copy of the repeat. *Coregonus peled*, *C. lavaretus*, and Eurasian and Beringian *C. clupeaformis* had two copies of the repeat, while the other North American *C. clupeaformis* had three copies, suggesting that *C. clupeaformis* of northwestern North America is a form of *C. lavaretus*.

Résumé : Les analyses phylogénétiques fondées sur les caractéristiques morphométriques, l'électrophorèse des protéines et l'ADN mitochondrial ont donné lieu à des hypothèses phylogénétiques contradictoires dans le cas de la sous-famille des *Coregoninae* (*Prosopium, Coregonus* et *Stenodus*). Pour résoudre ces contradictions, une analyse phylogénétique de treize taxons de *Coregoninae* a été réalisée à l'aide d'un gène nucléaire, le premier espaceur intertranscrits (ITS1) de l'ADN ribosomique (ADNr). Les séquences ITS1 de tous les ciscos d'Amérique du Nord étaient identiques. En accord avec les analyses fondées sur d'autres données moléculaires, le genre *Stenodus* a été inclus dans *Coregonus* et il n'y avait aucune base pour appuyer le monophylétisme des ciscos. L'analyse fondée sur l'ADNr a également classé *C. peled* comme l'espèce soeur de *C. lavaretus*, mais cette situation peut représenter un cas d'hybridation ou d'introgression. Un polymorphisme de longueur faisant intervenir une unité répétitive de 65–67 paires de bases a été trouvé dans le gène ITS1. Tous les ciscos, sauf *C. peled*, possédaient une copie de la séquence répétée. *Coregonus peled, C. lavaretus et C. clupeaformis* d'Eurasie et de Béring en avaient deux copies, tandis que l'autre *C. clupeaformis* nord-américain en avait trois, ce qui indiquerait que ces *C. clupeaformis* du nord-ouest de l'Amérique du Nord est une forme de *C. lavaretus*.
[Traduit par la Rédaction]

Introduction

The subfamily Coregoninae of the family Salmonidae contains three genera, *Coregonus*, *Prosopium*, and *Stenodus*. Two subgenera have been proposed within *Coregonus*: the subgenus *Coregonus* containing true whitefish with subterminal mouth, low gill raker number, and benthic feeding niche and the subgenus *Leucichthys* containing ciscoes with terminal or supraterminal mouth, high gill raker number, and pelagic feeding niche.

In Eurasia, the whitefish (*C. lavaretus*) underwent an extensive radiation after the retreat of the Pleistocene glaciers leaving behind a highly diverse complex of closely related species. In North America, *Coregonus artedi* and perhaps *C. zenithicus* radiated into the Great Lakes upon the retreat of the Wisconsin ice sheet. This radiation resulted in a diverse species flock of ciscoes endemic to the Great Lakes including *C. hoyi*, *C. kiyi*, and *C. nigripinnis* (Smith and Todd 1984).

The coregonines display ecophenotypic plasticity and have been problematic for taxonomists (Svärdson 1970; Lindsey 1981; Todd et al. 1981; Bodaly et al. 1991*b*; Shields and Underhill 1993). Extensive work has been done recently in an attempt to clarify the phylogenetic relationships of the coregonines. Phylogenetic analysis of this group has been done using osteological characters (Smith and Todd 1992), mitochondrial DNA (mtDNA) haplotype data (Bernatchez et al. 1991; Bernatchez and Dodson 1994), mtDNA sequence data (Lockwood et al. 1993), and allozyme electrophoresis (Bodaly et al. 1991*a*, 1994; Ermolenko 1992).

Based on these different data sets, five incongruitiies occur concerning the phylogenetic relationships of the species within the coregonines. These include (*i*) placement of the inconnu (*Stenodus leucichthys*), (*ii*) monophyly of the ciscoes, (*iii*) number of species comprising the ciscoes of the Laurentian Great Lakes, (*iv*) placement of *C. peled*, and (*v*) species-level status of *C. clupeaformis* and *C. lavaretus*.

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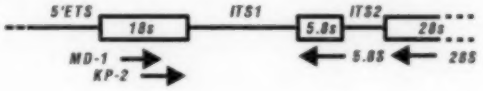
Fig. 1. Primers for PCR and sequencing of the transcribed spacers of the rDNA. Primers were designed from published sequences (references below). Sequences are as follows: MD-1 forward, 5′-CTT GAC TAT CTA GAG GAA GT-3′ (Salim and Maden 1981); KP-2 forward, 5′-AAA AAG CTT CCG TAG GTG AAC CTG CG-3′ (Salim and Maden 1981); 5.8S reverse, 5′-AGC TTG GTG CGT TCT TCA TCG A-3′ (Nazar and Roy 1978); 28S reverse, 5′-ATA TGC TTA AAT TCA GCG GG-3′ (Ware et al. 1983).



Of particular interest is the species designation of *C. clupeaformis* and *C. lavaretus*. Five mitochondrial races of *C. clupeaformis*, the lake whitefish of North America, have been identified (Bernatchez et al. 1991; Bernatchez and Dodson 1994). Each race is composed of a cluster of mtDNA haplotypes and was named for the glacial refugia in which it presumably differentiated. Those races west of the Great Divide of North America were designated as Beringian and Eurasian. Those east of the Great Divide were designated as Mississippian, Acadian, and Atlantic. The western races, Beringian and Eurasian, were found to be genetically more similar to *C. lavaretus* of Eurasia than to the *C. clupeaformis* eastern races, Mississippian, Acadian, and Atlantic. This suggests either one circumpolar species is present or the geographic distribution of the two species has been misinterpreted. Bodaly et al.'s (1991*a*, 1994) protein analysis concurs with the mtDNA haplotype analysis. However, the osteological character analysis of Smith and Todd (1992) found *C. clupeaformis* and *C. lavaretus* to be separate monophyletic groups.

In this study, the first internal transcribed spacer (ITS1) of the nuclear ribosomal DNA (rDNA) was sequenced to assess the phylogenetic relationship of members of the genus *Coregonus*. *Stenodus leucichthys* was included to assess its phylogenetic relationship to the genus *Coregonus*. *Prosopium cylindraceum* was included as the out-group taxon. The ITS1 of the rDNA has been successfully used in the phylogenetic analysis of several species including fishes of the genus *Salvelinus* (Pleyte et al. 1992; Phillips et al. 1994, 1995) and the genus *Oncorhynchus* (Domanico 1994). Other phylogenetic studies utilizing the rDNA ITS have been done with primates (Gonzales et al. 1990) and invertebrates (Vogler and DeSalle 1994; Fritz et al. 1994).

Materials and methods

Genomic DNA was obtained from the following cisco species: *C. albula* (Derwentwater, English Lake District, Great Britain), *C. laurettae* (Yukon River, Alaska, U.S.A.), *C. peled* (Finland, hatchery stock), *C. artedi* (Lake Nipigon, Ontario, Canada and Green Lake, Wisconsin, U.S.A.), *C. hoyi* (Lake Michigan, Wisconsin), *C. kiyi* (Lake Superior, Wisconsin), *C. zenithicus* (Lake Superior, Wisconsin), and *C. nigripinnis* (Lake Nipigon, Ontario, Canada). DNA was obtained from the following whitefishes: *C. nasus* (Mackenzie Delta, Northwest Territories, Canada), *C. lavaretus* (Loch Lomond, Scotland), *C. clupeaformis* (Lake Nipigon, Ontario, Canada), *Stenodus leucichthys* (Yukon River, Alaska, U.S.A.), and *Prosopium cylindraceum* (Lake Nipigon, Ontario, Canada).

Hearts and livers were frozen or preserved in 0.25 M EDTA – 20% DMSO buffer (pH 8.0). DNA was extracted using the Strategene kit (La Jolla, Calif.) or by phenol chloroform extraction (Sambrook et al. 1989).

The primers used for the polymerase chain reaction (PCR) (Saiki et al. 1988) and dideoxy sequencing are summarized in Fig. 1. Single-stranded DNA for sequencing was generated either by asymmetric PCR or by blunt end cloning into M13. Asymmetric PCR products were produced in two separate rounds of amplification. The primers MD-1 forward and 28S reverse were used in the initial PCR amplification to produce a DNA fragment spanning the ITS1 and ITS2 (second internal transcribed spacer). This fragment also includes the 5.8S rDNA. PCR reactions were 100 μL total volume. Each 100-μL reaction contained 2.5 units of *Taq* polymerase (Promega, Madison, Wis.); 10 μL of 10× $MgCl_2$-free buffer supplied by the manufacturer; 2 μL each of 1 mM dATP, dCTP, dTTP, and dGTP; 15 pmol of each primer; and 100 ng of genomic DNA. Magnesium chloride requirements differed among species. *Coregonus clupeaformis*, *C. lavaretus*, and *P. cylindraceum* required 4 μL of 25 mM $MgCl_2$ per 100-μL reaction. All other species required 6 μL 25 mM $MgCl_2$ per 100-μL reaction. Amplifications were carried out as follows in a Coy Model 60 thermal cycler or a Perkin Elmer thermal cycler model 480: 94°C for 1 min 30 s, 50°C for 2 min, 72°C for 3 min (36 cycles). The last cycle was programed with a 7-min extension at 72°C.

For the asymmetric PCR reactions, we used 5 μL of the initial round MD-1 forward – 28S reverse PCR product as template in 100-μL reactions. These reactions contained $MgCl_2$-free buffer, dNTPs, and $MgCl_2$ as described above. Primers for asymmetric products were 15 pmol MD-1 forward or 15 pmol 5.8S reverse primers each alone. Thermal cycling was the same as above.

Single-stranded asymmetric products were cleaned before sequencing. For each template to be sequenced, two 100-μL PCR reactions were combined and washed in a Millipore 30 000 NMWL filter (400 μL capacity; Millipore, Burlington, Mass.) five times with 300 μL sterile distilled water. After each addition of water, the filter was centrifuged at 1200 × *g*. The volume was reduced to <50 μL at the last spin.

Dideoxy sequencing was done using Sequenase 2.0 sequencing kit (U.S. Biochemical) according to instructions. Each sequencing reaction used 7 μL of the cleaned asymmetric single-stranded product. The MD-1 product was sequenced using the 5.8S reverse primer. The 5.8S product was sequenced using the KP-2 forward primer.

The MD-1 – 5.8S PCR product, as generated in the initial round of amplification above, was blunt end cloned into M13. Single-stranded DNA was sequenced (USB Sequenase) with the M13/pUC Forward Amplification Primer (5′-CCCAGTCACGACGTTGTAAAACG-3′) (Gibco BRL, Gaithersburg, Md.). Sequences of forward clones were extended using MD-1 forward or KP-2 forward primers for the forward clones and 5.8S reverse primer for the reverse clones. Sequencing was done from both strands to produce overlapping fragments of 44–156 bases.

DNA was sequenced from two individuals for *C. artedi*, *C. lavaretus*, *C. clupeaformis*, *S. leucichthys*, and *P. cylindraceum* and one individual for *C. albula*, *C. laurettae*, *C. kiyi*, *C. zenithicus*, *C. nigripinnis*, and *C. nasus*. *Coregonus laurettae*, *C. peled*, and *C. nasus* were sequenced from M13 clones (three or four each). The remaining species were sequenced from asymmetric PCR products.

Sequences were aligned visually using the ESEE 2.0 computer program (Cabot and Beckenbach 1989) to yield a minimum number of evolutionary steps with gaps inserted to maintain alignment. Aligned sequences gave a final data set of 749 base pairs (bp) (Fig. 2). Percent sequence divergence was determined by using the absolute genetic distances option in PAUP (Swofford 1993) (Table 1). Kimura two-parameter distances were calculated using MEGA (Kumar et al. 1993) (Table 2). All analyses were repeated with Jukes–Cantor distances yielding similar results.

Phylogenetic hypotheses were developed using two statistical models. PAUP, the maximum parsimony program of Swofford

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Fig. 2. Aligned sequence of the ITS1. Species abbreviations: P.cy., *P. cylindraceum*, S.le., *S. leucichthys*; C.al., *C. albula*; C.ar., *C. artedi*, *C. hoyi*, *C. zenithicus*, *C. kiyi*, *C. nigripinnis* (all sequences are identical); C.la., *C. laurettae*; C.cl., *C. clupeaformis*; C.lv., *C. lavaretus*; C.na., *C. nasus*; C.pe., *C. peled*. ?, missing data; *, phylogenetically informative sites, ·, invariant sites. Palindrome sequence is underlined, first copy in P.cy., second and third copies in C.cl. Dashes indicate sites not present in other species.

Table 1. Pairwise distances between taxa using absolute distance option of paup.

	1	2	3	4	5	6	7	8	9
1. *C. albula*	—	5.3 (4.1)	5.0 (4.1)	5.0 (3.6)	8.3 (6.4)	6.6 (5.3)	6.8 (5.7)	6.5 (5.5)	14.5 (12.2)
2. *C. artedi*	32 (26)	—	4.3 (3.8)	3.5 (2.8)	7.5 (6.4)	6.3 (5.9)	6.0 (5.7)	6.1 (6.0)	14.4 (12.7)
3. *S. leucichthys*	30 (24)	26 (22)	—	4.1 (3.3)	6.3 (5.2)	5.5 (4.6)	4.8 (4.5)	5.3 (4.8)	13.4 (12.4)
4. *C. laurettae*	30 (21)	21 (16)	—	25 (19)	4.6 (3.6)	3.8 (3.1)	3.5 (2.9)	3.6 (3.3)	13.8 (11.9)
5. *C. clupeaformis*	50 (37)	45 (37)	38 (30)	28 (21)	—	1.8 (1.2)	1.8 (1.0)	2.3 (1.4)	16.6 (14.5)
6. *C. lavaretus*	40 (31)	38 (34)	33 (27)	23 (18)	11 (7)	—	1.0 (0.5)	0.5 (0.2)	15.4 (13.6)
7. *C. nasus*	41 (33)	36 (33)	29 (26)	21 (17)	11 (6)	6 (3)	—	0.08 (0.7)	15.1 (13.8)
8. *C. peled*	39 (32)	37 (35)	32 (28)	22 (19)	14 (8)	3 (1)	5 (4)	—	15.3 (13.8)
9. *P. cylindraceum*	85 (71)	87 (74)	81 (72)	83 (69)	100 (84)	93 (79)	91 (80)	92 (80)	—

Note: Values above the diagonal are percent sequence divergence with the gaps in the runs retained. Values below the diagonal are the absolute distances with the gaps in the runs retained. Values in parentheses are the divergences and distances with the gaps in the runs omitted.

Table 2. Kimura two-parameter pairwise distances using the pairwise deletion option.

	1	2	3	4	5	6	7	8	9
1. *C.albula*	—	0.0338	0.0353	0.0299	0.0413	.0372	0.0373	0.0389	0.1239
2. *C. artedi*		—	0.0350	0.0242	0.0461	0.0442	0.0421	0.0456	0.1241
3. *S. leucichthys*			—	0.0348		0.0406	0.0385	0.0367	0.0402
4. *C. laurettae*				—	0.0207	0.0190	0.0171	0.0205	0.1214
5. *C. clupeaformis*					—	0.0046	0.0031	0.0061	0.1370
6. *C. lavaretus*						—	0.0015	0.0015	0.1324
7. *C. nasus*							—	0.0030	0.1294
8. *C. peled*									0.1330
9. *P. cylindraceum*									—

(1993), was used with the exhaustive search option. For the PAUP analysis, a maximum of 603 of the 749 aligned bases was used. Bases 1–4 of the aligned sequence were omitted as this was missing data in three species and lacked informative sites in the remaining six species. Bases 177–308 were omitted as the second and third copies of a palindrome. Each duplication was interpreted as a single evolutionary event. The presence of additional copies of the palindrome was coded as an additional character (0, 1). Bases 739–744 were omitted as a highly variable region with intraindividual differences in some species resulting from different numbers of As in the runs at the 3′ end. The data were bootstrapped 500 times retaining branches occurring at least 50% of the time.

Neighbor joining of Kimura two-parameter distances was used to construct distance trees (MEGA program of Kumar et al. 1993). The pairwise deletion of the gaps option was used and the data were bootstrapped 500 times.

All trees were rooted with *P. cylindraceum*, the least-derived species of the subfamily Coregoninae as determined by morphological (Norden 1961; Behnke 1972; Smith and Todd 1992) and genetic analysis (Bernatchez et al. 1988; Ermolenko 1992; Lockwood et al. 1993).

Additional individuals of *C. clupeaformis* and *C. lavaretus* were screened for rDNA ITS1 length variation using PCR amplification with the MD-1 forward – 5.8S reverse primer set. These additional samples were six *C. lavaretus*, Albe Hatchery, Czech Republic (Lake Pegipus, Poland, stock); one *C. lavaretus*, Hawswater, English Lake District, Great Britain; and six additional *C. clupeaformis* from Lake Nipigon, Ontario, Canada. DNA from three individuals for each of the five mitochondrial races of *C. clupeaformis* was provided by Dr. Louis Bernatchez, University of Laval, Quebec,Que.: Eurasian from Dezadeash Lake, southern Yukon; Beringian from Little Teslin Lake, southern Yukon; Mississippian from James Bay, Canada; Acadian from Lake Cliff, Canada (dwarf form); Atlantic from Lake Cliff, Canada (normal form). PCR products were electrophoresed in 2% agarose gels and visualized with ethidium bromide staining (Fig. 3).

Results

The sequence of the ITS1 (Fig. 2) was obtained for the ciscoes *C. albula* (590 bp), *C. laurettae* (598 bp), *C. peled* (668 bp), *C. artedi* (593 bp), *C. hoyi* (593 bp), *C. kiyi* (593 bp), *C. zenithicus* (593 bp), *C. nigripinnis* (593 bp); for the whitefish *C. nasus* (665 bp), *C. lavaretus* (666 bp), *C. clupeaformis* (725 bp); for the inconnu, *S. leucichthys* (601 bp); and for the round whitefish, *P. cylindraceum* (608 bp). For *C. artedi*, *C. lavaretus*, *C. clupeaformis*, and *S. leucichthys*, two individuals were sequenced, and no intraspecies variation was found. Intraindividual differences were found in *P. cylindraceum*, *C. peled*, and *C. laurettae* in the number of As in the runs at the 3′ end of the sequence. *Coregonus peled*, sequenced from M13 clones, had two clones in the 3′ orientation that differed by one A at position 739 of the aligned sequence. Three additional individuals of *P. cylindraceum*, which were not sequenced in their entirety, showed a similar addition or deletion in this region. In these individuals, sequencing gels from asymmetric PCR sequencing revealed bands offset by one base after base 739.

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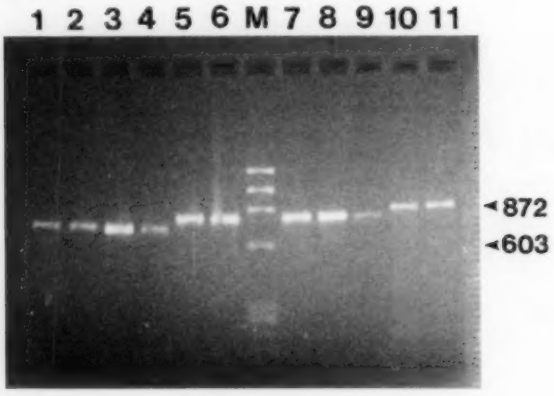
Fig. 3. Agarose gel of PCR products of the ITS1 of rDNA, ethidium bromide stained, illustrating the length polymorphism. One copy: lane 1, *P. cylindraceum*; lane 2, *S. leucichthys*; lane 3, *C. artedi*; lane 4, *C. albula*. Two copies: lane 5, *C. peled*; lane 6, *C. nasus*; lane 7, *C. lavaretus* (UK); lane 8, *C. lavaretus* (Poland). Three copies: lane 10, *C. clupeaformis* (James Bay); lane 11, *C. clupeaformis* (Ontario). Lane M, *Hae*III cut ϕX174 marker. Lengths given at right are in base pairs.



A length polymorphism resulting from a differing number of copies of a palindrome (65 bases in ciscoes, 66 or 67 bases in *C. clupeaformis* and *C. lavaretus*) was found (Fig. 3). One copy was found in North American ciscoes (*C. artedi*, *C. nigripinnis*, *C. hoyi*, *C. zenithicus*) and in *C. albula*, *C. laurettae*, *S. leucichthys*, and *P. cylindraceum*; two copies in *C. nasus*, *C. lavaretus*, *C. clupeaformis* (Eurasian and Beringian mtDNA races from Alaska), and *C. peled*; and three copies in the Mississippian, Atlantic, and Acadian mtDNA races of *C. clupeaformis*.

Transition/transversion ratios were determined for all variable sites within the in group including *S. leucichthys* in pairwise sequence comparisons of the aligned sequences (Table 3). No value was determined in six comparisons within the whitefish – *C. peled* group, where no transitions occurred. The range of values for the remaining comparisons was 0.6–1.25 except for *C. albula* and *S. leucichthys* with a lower value of 0.36. These values are similar to those found for the ITS1 and ITS2 within the genus *Oncorhynchus* (Domanico 1994).

We used two approaches to analyze aligned sequences with PAUP. In the first approach, gaps in runs (three or more of a base) were omitted, but bases inserted in runs were included. This approach is illustrated in Fig. 2, at site 75, where the gap in the run is omitted, but at site 76, where the inserted base is retained. This scheme gave 103 variable and 27 informative sites, which represents 26.2% of variable sites. The range of percent sequence divergence was 0.02–6.4% within the genus *Coregonus*. The sequence divergence between *S. leucichthys* and all coregonines ranged from 3.3 to 5.2%. These values are encompassed by the range of divergence within *Coregonus*.

In the second approach to phylogenetic analysis, gaps within runs were retained. This scheme resulted in 125 variable and 34 informative sites, which represents 27.2% of variable sites. Seven of the informative sites were gaps in runs. The range of the percent sequence divergence within *Coregonus* was 0.5–8.3%. The sequence divergence between *S. leucichthys* and all coregonines ranged from 4.1 to 6.3%. These values are encompassed by the range of divergence within *Coregonus*.

The topology of the bootstrapped trees (retaining branches found at least 50% of the time) from the two analyses differed only in the placement of *S. leucichthys* (Fig. 4). In the first analysis, with gaps in runs omitted, PAUP gave one best tree at 135 steps (Fig. 4*a*). The 50% bootstrapped tree placed *C. albula* basally and created a polytomy of *C. artedi* and *S. leucichthys* branching next on the tree. In the second analysis, (gaps in runs retained; Fig. 4*b*), PAUP produced two best trees at 164 steps. These trees differed only in the placement of *S. leucichthys*, which was either intermediate to *C. albula* and *C. artedi* or basal to all included coregonines. In the 50% bootstrapped tree, *S. leucichthys* formed a polytomy with *C. albula*. This polytomy was basal to the remaining coregonines.

All other relationships remained the same in both analyses. *Coregonus peled* was retained within the whitefish as a sister species to *C. lavaretus*, and a polytomy was created with *C. clupeaformis*, *C. nasus*, and the *C. lavaretus* – *C. peled* clade. *Coregonus laurettae* was basal to the whitefish clade.

Retention of the gaps in runs as informative sites (second analysis) tends to weaken the strength of the overall analysis as revealed by the consistency (CI) and retention index (RI) values (gaps in runs omitted: CI = 0.902, RI = 0.814; gaps in runs retained: CI = 0.878, RI = 0.756) and is the most conservative interpretation of the data set.

Bootstrap values were affected differently in the two analyses. With gaps in runs omitted, the branch defining the *C. lavaretus* plus *C. peled* clade from the remaining whitefish is strengthened from a value of 65 to 85. With the gaps in runs retained, the branch defining *C. laurettae* and the whitefish – *C. peled* clade is strengthened from a value of 67 to 86.

Percent sequence divergence was also affected. With gaps in runs retained (Table 1) the distances in all pairwise comparisons were increased (range 0.021–0.001) with the greatest increases between all coregonids including *S. leucichthys* and *P. cylindraceum*.

Kimura two-parameter distances calculated with the pairwise deletion option were used to construct neighbor-joining trees with MEGA. A range of distances of 0.0015–0.0461 was obtained within the genus *Coregonus*. The range of distances between *S. leucichthys* and all members of the genus *Coregonus* was 0.0348–0.0406 (Table 2). This range of distances is within the range of distances among members of the genus *Coregonus*. The resulting phylogeny (Fig. 5) maintained the *C. laurettae* whitefish branch and resolved the whitefish polytomy with *C. nasus* basal. *Coregonus clupeaformis* branched next with the *C. lavaretus* – *C. peled* clade being most derived at a bootstrap value above 50%. *Stenodus leucichthys* was basal with *C. albula* and *C. artedi* branching in that order. However, the bootstrap values were <50% at these nodes indicating a tree that retains branches found at least 50% of the time would place these three species as a basal polytomy.

Parsimony and neighbor-joining phylogenies produced very similar tree topologies. These topologies agreed in the sister relationship of *C. peled* and *C. lavaretus* and the close relationship of *C. nasus* and *C. clupeaformis* (Mississippi race). Neither parsimony or neighbor-joining were able to resolve the branching order of *C. artedi* (GL), *S. leucichthys*, and *C. albula*.

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Table 3. Transition/transversion data.

	1	2	3	4	5	6	7
1. *C. albula*	—	0.600	0.360	0.875	0.690	0.667	0.818
2. *C. artedi*	6/10	—	0.667	0.667	0.692	0.750	0.818
3. *S. leucichthys*	4/11	6/9	—	0.857	0.545	0.600	0.667
4. *C. laurettae*	7/8	4/6	6/7	—	0.833	1.000	1.250
5. *C. clupeaformis*	9/13	9/13	6/11	5/6	—	—	—
6. *c. lavaretyus*	8/12	9/12	6/10	5/5	0/3	—	—
7. *C. nasus*	9/11	9/11	6/9	5/4	0/2	0/1	—
8. *C. peled*	8/12	9/13	6/11	6/5	0/4	0/1	0/2
9. *P. cylindraceum*	27/29	28/29	23/29	27/27	29/32	29/31	29/30

Note: Values above the diagonal are the transition/transversion ratios. First and second values below the diagonal are the numbers of transitions and transversions, respectively. nd, not determined (when transitions are 0).

Discussion

Five incongruities are evident between the phylogenies (Figs. 6*a*–6*e*) generated by previous analyses. These include (*i*) the placement of the inconnu *S. leucichthys*, (*ii*) the monophyly of the ciscoes, (*iii*) the number of species comprising the ciscoes of the Laurentian Great Lakes, (*iv*) the placement of *C. peled*, and (*v*) the species-level status of *C. clupeaformis* and *C. lavaretus*. The inclusion of a nuclear DNA data set, the rDNA ITS1 spacer sequence, has helped to resolve some problems but has produced new questions.

Placement of inconnu

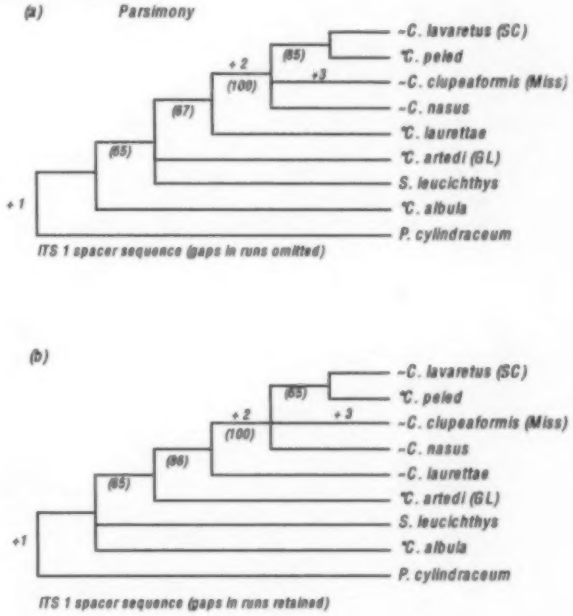
The inclusion of *S. leucichthys* within the genus *Coregonus* is supported by the phylogenetic analysis of Smith and Todd (1992) (Fig. 6*a*), Bodaly et al. (1991*a*) (Fig. 6*d*), and Bernatchez et al. (1991) (Fig. 6*b*) and refuted by Lockwood et al. (1993) (Fig. 6*c*) and Ermolenko (1992) (Fig. 6*e*).

Although the placement of the genus *Stenodus* within the genus *Coregonus* is unresolved in the phylogenies based on rDNA ITS1 sequence data, distance data support the inclusion of *S. leucichthys* within *Coregonus*. Percent sequence divergence from PAUP (Table 1) and Kimura two-parameter distance (Table 2), suggest *S. leucichthys* is as closely related to members of the genus *Coregonus* as species traditionally considered within *Coregonus* are to one another. The range of Kimura two-parameter distances (0.0348–0.0406) and mean distances from PAUP (0.003–0.011) for *S. leucichthys* from all coregonines fall within the range of Kimura two-parameter distances (0.0015–0.0461) and the mean distances from PAUP (0.001–0.019) among all members of the genus *Coregonus* included in this study. The presence of naturally occurring hybrids of *S. leucichthys* and members of the genus *Coregonus* (Berg 1948; Alt 1971; Reist et al. 1992) provides further evidence for a close relationship between these two genera. The use of the name *Stenodus* as a subgeneus designation for *Coregonus* (*Stenodus*) *leucichthys* was suggested by Smith and Todd (1992). The distance data produced by this study strongly supports this designation of *S. leucichthys* as belonging within the genus *Coregonus*.

Monophyly of the ciscoes

The monophyletic relationship of the ciscoes is supported by Smith and Todd (1992) (Fig. 6*a*) but not by Bodaly et al. (1991*a*) (Fig. 6*d*), Bernatchez et al. (1991) (Fig. 6*b*), Lockwood et al. (1993) (Fig. 6*c*), and Ermolenko (1992) (Fig. 6*e*).

Fig. 4. PAUP parsimony bootstrapped trees retaining branches found at least 50% of the time: (*a*) gaps in runs omitted (CI = 0.902, RI = 0.814); (*b*) gaps in runs included (CI = 0.878, RI = 0.756). Percent bootstrap values are given in parentheses. The values +1, +2, and +3 indicate the number of palindrome copies. *, Individuals classified as ciscoes; ~, individuals classified as whitefish. Place abbreviations: SC, Scotland; Miss, Mississippian; GL, Great Lakes.



Placement of the ciscoes within the subgenus *Leucichthys* is not supported by the rDNA sequence data, as the ciscoes did not form a monophyletic group. In both the parsimony (Fig. 4) and neighbor-joining (Fig. 5) phylogenies, either *C. albula* or *S. leucichthys* is on the basal branch with the ciscoes branching sequentially. In addition, *C. peled*, considered a cisco, falls within the group representing the true whitefish, further deviating from monophyly. If the view is held that taxonomic nomenclature should describe evolutionary events, then the use of the subgenus name *Leucichthys* for the cisco group is not appropriate and should be discontinued.

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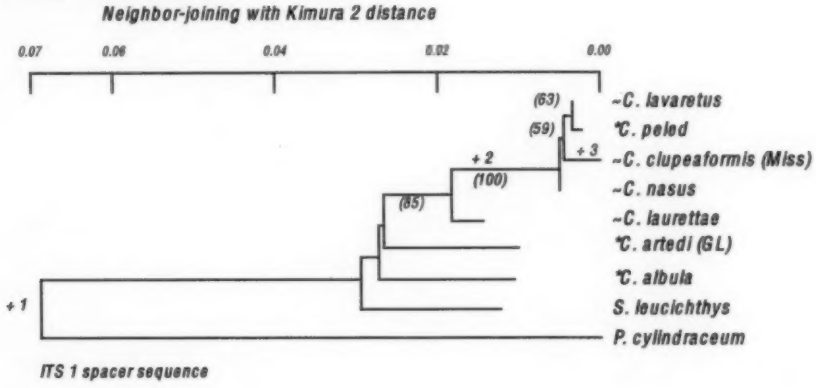
Fig. 5. MEGA neighbor-joining tree with Kimura two-parameter distances. Percentage bootstrap values are given in parentheses. The values +1, +2, and +3 indicate the number of palindrome copies. *, Individuals classified as ciscoes; ~, individuals classified as whitefish. Place abbreviations: Miss, Mississippian; GL, Great Lakes.



Number of cisco species in the Great Lakes

There is no evidence from this study to support pre-Wisconsin glacial differentiation of the North American ciscoes *C. artedi* and *C. zenithicus* as suggested by Smith and Todd (1984) or to establish them as separate species if possession of unique DNA sequences is used as the criterion. The phylogenetic analysis of the Great Lakes ciscoes by Smith and Todd (1992) was based on osteological characters. This phylogenetic hypothesis (Fig. 6*a*) distinguishes three groups of Great Lakes (GL) ciscoes, the *C. artedi* (GL) complex (*C. artedi*, *C. reighardi*, *C. zenithicus*, *C. johannae*) with *C. hoyi* and *C. kiyi* as two separate taxa. However, the phylogeny of Bernatchez et al. (1991) (Fig. 6*b*) based on mtDNA haplotypes was unable to distinguish *C. artedi* and *C. hoyi*.

Additional evidence for recent divergence among the ciscoes was found by Snyder et al. (1992) who surveyed the mtDNA haplotypes of *C. artedi* from inland lakes and Lake Superior and *C. hoyi* from Lake Superior. Snyder et al. could not distinguish *C. artedi* from *C. hoyi* in single collection samples. However, Snyder et al. argue that reproductive isolation is likely based on a 100-bp insertion haplotype present in Lake Superior and inland *C. artedi*, which is absent in *C. hoyi*.

Sajdak (1995) found further evidence for the very recent divergence of the Great Lakes ciscoes in multiple 5S ribosomal DNA spacer sequences from each of two *C. artedi* (Green Lake, Wisconsin, and Lake Nipigon, Ontario) and two *C. zenithicus* (Lake Nipigon, Ontario). All 5S spacer sequences were unique. Phylogenetic hypotheses generated in PAUP consistently intermingled sequences from *C. artedi* and *C. zenithicus*. The sequences from *C. artedi* (Green Lake) clustered with sequences from individuals of both species from Lake Nipigon.

In the present study, the rDNA sequences of the North American ciscoes (*C. artedi*, *C. zenithicus*, *C. hoyi*, *C. kiyi*, *C. nigripinnis*) were identical. The current evidence, therefore, indicates that the Great Lakes cisco group is an incipient species group (Smith and Todd 1984) whose differentiation is driven by trophic adaptation and reproductive isolation (T. Ehlingher, University of Wisconsin, Milwaukee, personal communication).

Placement of *Coregonus peled*

There is general agreement on the placement of *C. peled* within a cisco clade with *C. albula* and *C. laurettae* (Smith and Todd 1992 (Fig. 6*a*); Bernatchez et al. 1991 (Fig. 6*b*); Bodaly et al. 1991*a* (Fig. 6*d*)). Only Ermolanko (1992) (Fig. 6*e*) places *C. peled* as a sister taxon to whitefish. The *C. peled* used in this study is one of the same individuals used by Bernatchez et al. (1991) in his mtDNA analysis, which placed *C. peled* with *C. albula* and *C. sardinella*. In the present study, analysis of rDNA sequence placed *C. peled* not as a sister species of *C. albula* within the ciscoes as expected but as a sister species to *C. lavaretus* within the whitefishes. This finding is surprising in light of other molecular distance data. Bernatchez et al. (1991) calculated percent sequence divergence based on mtDNA haplotypes. They found the percent sequence divergence of *C. peled* from *C. albula* to be 1.00%, from *C. sardinella* to be 1.06%, and from *C. lavaretus* to be 1.19%, placing *C. peled* within the ciscoes. Bodaly et al. (1994) calculated a Nei unbiased genetic distance from allozyme data. They found the genetic distance of *C. peled* from *C. albula* to be 0.103, from *C. sardinella* to be 0.161, and from *C. lavaretus* to be 0.181. Therefore, the closest relatives of *C. peled* were estimated to be *C. albula* and *C. sardinella*. This also places *C. peled* close to the cisco group. However, *C. lavaretus* was next closest to *C. peled* in both studies (Bernatchez et al. 1991; Bodaly et al. 1994).

Interfertility of *C. peled* with *C. lavaretus* could account for the introgression of *C. lavaretus* rDNA into *C. peled* populations. Berg (1948) reported that N.A. Ostroumov found hybrids of *C. peled* and whitefish in the Pechora River, Siberia, indicating interfertility. Luczynski et al. (1992) reported extensive hybridization between European *C. lavaretus* and *C. peled* in Polish lakes after *C. peled* introductions. *Coregonus peled* is confined to a small range in Siberia, and populations are fragmented because this species is lacustrine, leaving lakes only to spawn a short distance up the adjacent rivers (Berg 1948). The *C. peled* individual used in this study is from a hatchery in Finland. The founding stock may have come from a population that previously hybridized with *C. lavaretus*, or hybridization may have occurred in the hatchery. If so, the rDNA of *C. lavaretus*, which is subject to gene

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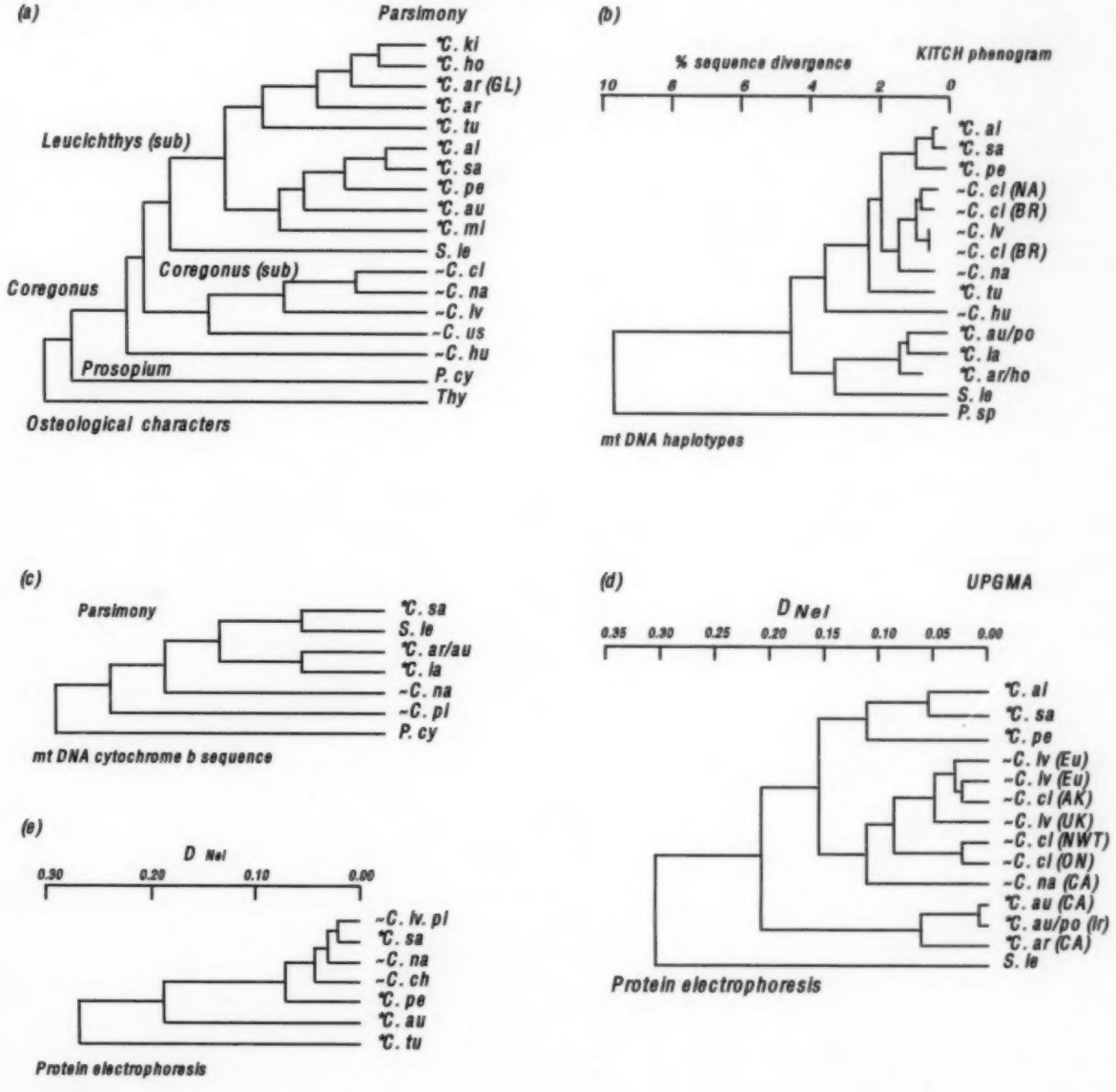
Fig. 6. Phylogenetic hypotheses of other investigators: (*a*) after Smith and Todd (1992); (*b*) after Bernatchez et al. (1991); (*c*) after Lockwood et al (1993); (*d*) after Bodaly et al (1991*a*); (*e*) after Ermolenko (1992). *, Individuals classified as ciscoes; ~, individuals classified as whitefish. Species abbreviations: P.cy., *P. cylindraceum*, S.le., *S. leucichthys*; C.al., *C. albula*; C.ar., *C. artedi*; C.la., *C. laurettae*; C.cl., *C. clupeaformis*; C.lv., *C. lavaretus*; C.na., *C. nasus*, C.pe., *C. peled*; C.ki., *C. kiyi*; C.ho., *C. hoyi*; C.tu., *C. tugun*; C.sa., *C. sardinella*; C.au., *C. autumnalis*; C.mi., *C. migratorius*; C.us., *C. ussuriensis*; C.hu., *C. huntsmani*; Thy, *Thymallus*; C.po, *C. pollan*; C.pi, *C. pidschian*; C.ch., *C. chadary*. Place abbreviations: GL, Great Lakes; NA, North America; BR, Beringian; Eu, Europe; AK, Alaska; UK, United Kingdom; NWT, Northwest Territories; ON, Ontario; CA, Canada; Ir, Ireland.



conversion (Hillis et al. 1991), may have replaced the typical cisco rDNA in this particular stock.

The sequence data suggest that convergent evolution can be ruled out. Because the palindrome is duplicated in its entirety, the structure may contribute to its ability to be duplicated. If this were true, the duplication event could have occurred independently in *C. peled*. This seems unlikely. The *C. peled* sequence from base 109 to 241 (which includes the first and second copies of the palindrome) is identical to the whitefish (*C. clupeaformis*, *C. nasus*, and *C. lavaretus*). However, the first repeat of the palindrome has fixed differences at bases 111 and 124, which distinguish the ciscoes (*C. albula*, *C. artedi*, and *C. lauretta*) from the whitefish. The second repeat of the palindrome is unlike the first, having the cisco sequence at the base corresponding to base 111, the whitefish sequence at the base corresponding to base 124, and a unique

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base at base 179, which is a G at base 112, the corresponding base within the first repeat. In summary, the second repeat differs from the first and is identical in all species sequenced. This would indicate that the duplication is a single evolutionary event.

Species-level status of lake whitefish

Coregonus lavaretus and *C. clupeaformis* have been divided into two taxonomic units based on continental distribution, with *C. lavaretus* in Europe and Asia and *C. clupeaformis* in North America. This demarcation is supported by Smith and Todd's (1992) phylogeny (Fig. 6*a*) using osteological characters but is challenged by recent molecular data. The phylogenies based on mtDNA haplotypes (Bernatchez et al. 1991; Bernatchez and Dodson 1994) (Fig. 6*b*) and allozyme electrophoretic analysis (Bodaly et al. 1991*a*; Bodaly et al. 1994) (Fig. 6*d*) group *C. clupeaformis* of Alaska and the Yukon, designated as Beringian and Eurasian races, with *C. lavaretus* of Eurasia. The rDNA evidence from this study supports the inclusion of the Beringian and Eurasian races of *C. clupeaformis* with *C. lavaretus*. These two races each have two copies of the ITS1 palindrome, while all *C. clupeaformis* from the Mississippian, Atlantic, and Acadian races have three copies.

The congruent molecular data (Bernatchez et al. 1991; Bernatchez and Dodson 1994; Bodaly et al. 1991*a*) and the rDNA sequence data reported here suggest two possible resolutions for whitefish taxonomy. The Alaska–Yukon populations of *C. clupeaformis* could be recognized as *C. lavaretus* and the Mississippian, Atlantic, and Acadian races as *C. clupeaformis*, possibly a subspecies. Conversely, as Bernatchez and Dodson (1994, p. 249) suggest, "*C. lavaretus* and *C. clupeaformis* should be recognized as a single, variable and circumpolar ranging species, *Coregonus lavaretus*, according to the most ancient name in use."

Unique placement of *C. albula*

Placement of *C. albula* as the earliest branching of the ciscoes is unique to this study. Bernatchez et al. (1991) and Bodaly et al. (1991*a*) place the *C. artedi* clade branching first with *C. albula* in a sister clade to the *C. lavaretus* – *C. nasus* clade. Smith and Todd (1992) place the *C. lavaretus* – *C. nasus* clade as branching first with *C. albula* as a sister clade to the *C. artedi* clade.

The evolutionary history of the subfamily Coregoninae is complicated by biotically and abiotically induced morphological plasticity, hybridization, and introgression. Smith and Todd (1992) concluded that some of the homoplasy found in the osteological character data is probably due to convergent evolution, but homoplasy may also be a consequence of extensive introgression resulting from hybridization among coregonine species. Additional evidence for the introgression of mtDNA is reported by S.E. Hartley (Department of Biological and Molecular Science, University of Sterling, Scotland, U.K., personal communication) who found mtDNA haplotypes of *C. albula* from the Lake District of England (LD UK) to be closer to *C. lavaretus* (LD UK), than *C. lavaretus* (LD UK) was to *C. lavaretus* populations of Scotland and Wales. The possibility of extensive introgression among coregonines is also supported by rDNA evidence for *C. peled* presented in this study. Morphological and mtDNA criteria define the *C. peled* individual used in this study as a cisco, yet it contains rDNA possibly derived from *C. lavaretus*.

Introgression and hybridization have played a role in coregonid evolution. Therefore, several strategies should be adopted in the study of coregonine systematics. First, groups of species from biogeographically significant areas, which could have undergone hybridization and introgression, should be included in study samples, as Snyder et al. (1992) did.

Second, molecular methods provide the genetic markers necessary to examine the evolution of genomes and species in great detail. Svärdson (1970) suggests that morphological and protein characters may not allow the recognition of hybrids after only a few generations of backcrossing and suggests the use of several independent genetic markers. Smith and Todd (1992) conclude that introgression has increased homoplasy in allozyme data, making them unreliable. Therefore, additional molecular methods should be used in conjunction with detailed morphological analysis.

When looking for evidence of hybridization and introgression, the most reliable results will be obtained if the same individual is examined for morphology and multiple mtDNA and nuclear DNA markers. Therefore, the coregonines are an ideal group for the "new museum collection," which will contain not only the voucher specimen carefully preserved but also tissue (perhaps scales and otoliths) and DNA. As they become available, new genetic markers can then be applied to previously studied specimens.

In conclusion, molecular data from both mtDNA and nuclear rDNA support the inclusion of *Stenodus* in *Coregonus*, the paraphyly of the ciscoes, and the Great Lakes ciscoes as incipient species. These data also support the inclusion of the Alaskan populations of *C. clupeaformis* as *C. lavaretus* with the Mississipian and Atlantic populations as either subpecies or sister species. Molecular data also provides evidence for hybridization and introgression between sympatric species. This means that construction of an accurate phylogeny of coregonines will require combined morphological and molecular studies on the same specimens. It is very important that future researchers preserve both tissue for DNA analysis and voucher specimens for morphological analysis.

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The relationship between prey selectivity and growth and survival in a larval fish

Christine M. Mayer and David H. Wahl

Abstract: We examined prey preference, growth, and survival of small larval (8–10 mm total length (TL)), large larval (11–17 mm TL), and early juvenile (>18 mm TL) walleye (*Stizostedion vitreum*) in laboratory aquaria and field mesocosms using multiple prey assemblages that included cladoceran, copepod, and rotifer prey of varied sizes. Both prey taxa and size affected prey preference during the larval period. All sizes of walleye avoided rotifer and nauplii prey. Small and large larvae selected for intermediate-sized (0.4–0.9 mm) cladoceran prey and selected against large prey (>0.9 mm) of both taxa. Although neither capture efficiency nor handling time differed between prey taxa, larvae oriented more frequently towards cladoceran prey suggesting that they were more visible than copepods to these small fish. Larval walleye that were fed exclusively cladoceran prey survived better than fish that were fed other prey. Early juveniles selected primarily on the basis of prey size, choosing large copepods and cladocerans. Prey taxa did not affect early juvenile growth or survival. Prey taxa and prey size interacted with predator size to influence selectivity and its effect on growth and survival. Consequently, these factors must be considered in combination when examining the importance of foraging decisions in young fish.

Résumé : Nous avons examiné la préférence alimentaire, la croissance et la survie de dorés jaunes (*Stizostedion vitreum*) larvaires de petite taille (8–10 mm LT), de grande taille (11–17 mm LT) et au début du stade juvénile (>18 mm LT) dans des aquariums en laboratoire et dans des mésocosmes naturels en utilisant des combinaisons de proies multiples comprenant des cladocères, des copépodes et des rotifères de différentes tailles. Le taxon et la taille de la proie ont tous deux influé sur la préférence alimentaire durant la période larvaire. Les dorés jaunes de toutes les tailles ont évité les proies constituées de rotifères et de nauplii. Les larves de petite et grande taille choisissaient les cladocères de taille intermédiaire (0,4–0,9 mm) et négligeaient les proies de grande taille (>0,9 mm) des deux taxons. Bien que ni l'efficacité de capture ni le temps de manipulation n'aient différé entre les taxons de proie, les larves s'orientaient plus fréquemment vers les cladocères, ce qui indiquerait que ces derniers sont plus visibles que les copépodes pour ces petits poissons. Les dorés jaunes larvaires qui ont été alimentés exclusivement de cladocères ont eu une meilleure survie que les poissons qui ont été nourris avec d'autres proies. Les juvéniles précoces ont exercé un choix fondé principalement sur la taille de la proie, préférant les copépodes et les cladocères de grande taille. Les taxons de proie n'ont pas eu d'effets sur la croissance ni sur la survie des juvéniles précoces. Le taxon et la taille des proies ont interagi avec la taille du prédateur pour influer sur la préférence et ses effets sur la croissance et la survie. Par conséquent, ces facteurs doivent être pris en considération en même temps lorsqu'on examine l'importance des décisions de broutage chez le jeune poisson.
[Traduit par la Rédaction]

Introduction

Planktivorous fish feed selectively, often choosing large prey items, and driving the structure of zooplankton populations towards smaller taxa or smaller individuals within a taxon (Brooks and Dodson 1965). Young fish are less likely than adults to choose the largest available prey items (Hansen and Wahl 1981; Mills et al. 1984; Bence and Murdoch 1986; Confer et al. 1990), and preferred prey size increases with fish size (Miller et al. 1990; Schael et al. 1991). Zooplankton behavior also affects selectivity; the ability of copepods to dart rapidly reduces their probability of capture relative to cladocerans (Drenner et al. 1978; Mills et al. 1984). However, in some cases, copepods are selected despite their greater evasive ability and small size (Confer et al. 1990). Aspects of prey morphology, such as shape differences associated with taxon, alterations to prey shape by spines (O'Brien et al. 1979), and prey coloration (Zaret 1980), may affect selective feeding by fish. Both adult (Werner and Hall 1974; Bartell 1982) and young planktivores (Confer et al. 1990) may feed more selectively at high prey density, although this may depend on other conditions such as light level (Mills et al. 1986). Zooplankton distribution is frequently patchy (see Malone and McQueen 1983 for review), so that planktivores may experience great variation in the number of available prey. Multiple factors such as prey morphology, behavior, and density as well as predator size may influence selective predation by planktivores.

The relative importance of factors affecting selective feeding and their interaction is less well understood in young fish than in adults. Several reasons may be responsible for an apparent lack of general patterns of selective feeding in young fish. For example, young walleye (*Stizostedion vitreum*) have been reported to select prey based solely on size (Merna 1977; Hokanson and Lien 1986), to select either cladocerans (Raisanen and Applegate 1983) or copepods (Fox 1990; Graham and Sprules 1992), and to avoid cladocerans (Houde 1967).

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Often, a single attribute of the prey or predator has been examined, thereby ignoring the potentially interactive effects of traits such as prey taxon and size. Even when multiple factors are examined, fish have been presented with a single prey assemblage, limiting generalization. Also, studies that do not consider fish size may produce confounding results as fish have been shown to switch preference as they grow (Michaletz et al. 1987; Jackson et al. 1992). To understand the relative importance of the characteristics of predator and prey in determining prey choice several factors must be considered using multiple prey assemblages and a range of fish sizes.

Selective feeding is an evolutionarily selected tendency to maximize energy intake per unit time (Schoener 1987) and, thus, should improve the predator's growth and survival given a particular prey assemblage. Prey selectivity may influence larval fish growth and survival. Not all prey assemblages will allow maximum potential growth, but for a particular assemblage (density, species, size), the preferred prey should allow the fastest growth. Diet composition can affect growth rates of young fish (Gophen 1980) and may vary with prey density, size, and species of prey. Young fish may grow faster when consuming copepods (Lemly and Dimmick 1982; Confer and Lake 1987) or cladocerans (Mills et al. 1989*a*; Adeyemo et al. 1994). Zooplankton size may also influence growth (Mills et al. 1989*a*, 1989*b*). Zooplankton assemblage affected fish growth, and the effect depended on the size of the fish (Miller et al. 1990). Some of these studies presented prey individually, while others correlated growth to prey items from heterogeneous prey assemblages. Therefore, it is difficult to separate the effects of presence of single or multiple prey on selectivity and growth or to discern any indirect effects of concurrent presentation, such as interference. We know of no studies that have documented selection and subsequently shown if the selected prey allowed better growth or survival.

This work is an attempt to elucidate the combined effects of larval size, prey size, prey taxa, and prey density on the foraging, growth, and survival of very young fish. We began by asking if young walleye, which are visually feeding planktivores, selected for particular taxa or sizes of prey and if prey density affected preference. Secondly, we asked if young walleye do select a particular prey, do these prey confer faster growth or better survival. Finally, we wished to know which aspects of the predator–prey encounter help explain selective prey choice. We conducted laboratory and field mesocosm experiments to test for selective feeding across a range of young fish sizes. We subsequently tested the effect of prey type on growth and survival. In addition, we observed foraging behaviors of larval fish to examine the mechanisms that underlie differential responses to prey.

Materials and methods

We chose three size-classes of walleye to represent different levels of behavioral and somatic development (Balon 1975; Li and Mathias 1982): small larvae (8–10 mm total length (TL)), large larvae (11–18 mm TL), and early juveniles (>20 mm TL). All laboratory experiments were conducted in 30-L glass aquaria unless otherwise noted. Laboratory cultures and field collections provided cladoceran and copepod prey. Rotifers and copepod nauplii, either field collected or grown in cultures, were filtered through a 125-μm sieve. Three zooplankton subsamples were counted to determine density and added volumetrically to aquaria to produce the desired densities.

At the end of each selective feeding experiment, all fish were measured (nearest 0.01 mm) and preserved in 70% ethanol. Prey from the entire gut of each fish were counted and identified to the lowest possible taxon, mostly to genus, but in some cases to family (e.g., Chydoridae). Zooplankton total length, excluding spines and setae, was measured (nearest 0.01 mm) using a dissecting microscope and a digitizing tablet viewed through a drawing tube. All zooplankton remaining after feeding were preserved in sugar–formalin and measured in the same manner as the gut samples.

Selective feeding in field enclosures

Experiments were conducted in waters in east-central Illinois, which contained a range of zooplankton assemblages. These included a rotifer-dominated pond, two experimental ponds, one small reservoir (Ridge Lake), and two locations in a large reservoir (Lake Shelbyville). Floating plastic enclosures (121 L) were gently filled with lake water and either 25 small or large larvae, or 3 early juveniles, were allowed to feed for 24 h. Lower numbers of early juvenile fish ensured that no significant prey depletion occurred. Prior to each experiment we preserved a 30-L zooplankton subsample from each enclosure by filtering through a 63 μm mesh sieve. At the end of the feeding period all remaining zooplankton and walleye in the enclosures were preserved.

In the laboratory, initial zooplankton subsamples were counted and identified. The entire subsample was counted unless numbers were too high, in which case three to five 1-mL aliquots were counted until 200 organisms of each taxon had been counted (Dettmers and Stein 1992). The entire subsample was examined for rare taxa. Size distributions were determined by measuring at least 30 individuals of each taxon from the 30-L subsample. Prey organisms were identified, counted, and measured from guts of a subsample of fish (10 larvae, 3 early juveniles, or all survivors) from each enclosure.

Prey selectivity was estimated by calculating Chesson's coefficient of selectivity, α, which is determined by the maximum likelihood estimator:

$$\alpha_i = \frac{r_i / p_i}{\sum_{i=1}^{m} (r_i / p_i)}$$

where r_i is the proportion of items of food type i in the predator's diet, and p_i is the proportion of food type i in the environment (Chesson 1983). Prey were assigned to six categories ($m = 6$) consisting of three size-classes (<0.4, 0.4–0.9, and >0.9 mm length) within each taxonomic group (cladocerans and copepods). Values of α_i from individual fish were averaged for each enclosure, our experimental unit.

All selectivity coefficients were compared with random feeding (1/number of prey types) using paired t tests with a Bonnferoni correction of alpha level (0.05/number of pairwise comparisons) to provide an overall error rate of 0.05 (Scheiner 1993). The data were arcsine transformed to meet the assumption of normality. The relationship of prey density to coefficients of selectivity was examined separately, using correlation analysis.

Selective feeding in the laboratory

All size-classes of walleye (10 small or large larvae, 1 early juvenile) were fed for 1 h in aquaria held in an environmental chamber at 20°C. Seven macrozooplankton densities were replicated in separate aquaria ($N = 3$/treatment, total aquaria = 21). Zooplankton collected from nearby lakes with a 156 μm mesh, 0.5 m diameter zooplankton net provided a natural prey assemblage. We manipulated the prey density in each aquarium by adding prey such that cladoceran densities were 5, 25, 50, 75, 100, 200, or 300·L^{-1}, although this resulted in slightly different total zooplankton densities between experiments. Taxonomic composition, size distribution, and abundance of the lake zooplankton were determined as previously described. Coefficients of selectivity for individual fish were calculated as described previously, and means of these values were calculated for each tank, our

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experimental unit. As in field enclosure experiments the effect of prey density on coefficients of selectivity was tested separately. Unlike the field experiments, density was controlled in the laboratory; therefore, we used regression analysis with prey density as the independent variable and coefficient of selectivity as the dependent variable (Sokal and Rohlf 1981).

Growth and survival

We examined the effects of prey taxon and density on walleye growth and survival in aquaria held in an environmental chamber (20°C and 12 h light : 12 h dark photoperiod). Fish of a single size-class ($N = 50$ small larvae, $N = 30$ large larvae, or $N = 8$ early juveniles) fed on controlled prey assemblages. Initial attempts to measure individual walleye prior to these experiments resulted in injury. To assure that fish in all aquaria were similar in size, we randomly assigned them to aquaria, adding them from a common holding tank sequentially to avoid concentrating slower or faster swimming fish. Prey treatments were either rotifers and nauplii (>500·L^{-1}), *Daphnia* spp. alone (50·L^{-1}), copepods alone (50·L^{-1}), *Daphnia* spp. (25·L^{-1}) and copepods (25·L^{-1}), or *Daphnia* spp. (50·L^{-1}) and copepods (50·L^{-1}) and were replicated in three aquaria per treatment. Prey were not size sorted in this first experiment. This design allowed us to compare fish fed equal numbers of copepod or cladoceran prey with and without the presence of the other taxa and to evaluate if the presence of one prey type interfered with consumption of the other. We maintained zooplankton densities throughout the experiment (5 days for larvae, 7 days for juveniles) by subsampling prey with a clear Plexiglas tube lowered onto a rubber stopper on the tank bottom, and adjusted densities by adding the appropriate volume from a stock culture. At the end of each experiment all surviving fish were measured (TL, nearest 0.01 mm).

A parallel experiment assessed the effects of prey taxon and size on walleye growth. *Daphnia* spp. and copepods were each passed through a 500-µm sieve to create a small and a large size-class. Zooplankton retained on the sieve corresponded to the large category in the selectivity experiments, and those that passed through corresponded to the small and medium categories combined (<0.9 mm). Thirty large larvae fed on either small copepod, small *Daphnia* spp., large copepod, or large *Daphnia* spp. for 5 days. Prey densities were maintained at 50·L^{-1} as previously described. For all growth and survival experiments, mean lengths and survival from three replicate tanks per treatment were compared using multivariate analysis of variance tests (MANOVA) and multiple comparison tests ($\alpha = 0.05$). Survival proportions were arcsine transformed prior to analyses.

Foraging behavior and morphometric comparisons

We quantified foraging behavior by placing a single walleye (either a large larva or early juvenile) in a circular behavioral arena (2 L) with opaque sides and open top. Fish were acclimated to the arenas without food for at least 8 h. The numbers of occurrences and total time spent inactive, orienting, striking, and capturing prey were recorded using a Datamyte event recorder (Electro/General Corporation, Minnetonka, Minn.). Ten prey, either small or large copepods or cladocerans, were introduced at the beginning of each 30-min experiment (five replicates per treatment for large larvae, nine for early juveniles). Orientation was designated as the time during which the predator directed its head toward an individual prey item. Reactive and strike distances were measured in a subset of experiments (6 for large larvae, 14 for early juveniles). Reactive distance was the distance at which the predator oriented toward a prey item; strike distance was the distance that the fish moved towards prey at burst speed. Distances were estimated by comparison to a grid on the bottom of the arena (10-mm increments). Handling time, defined as the time between initial capture and resumption of swimming, was recorded for all successful captures. Responses to prey size and taxa for all behaviors were compared using analysis of variance and Tukey's multiple comparison test ($\alpha = 0.05$).

Mouth gapes of walleye were measured using machined cones of incremental dimensions (Arts and Evans 1987). Cones were inserted into the mouth until the opercula flared, representing the point to which a fish can naturally open its mouth. A measurement was taken from a micrometer attached to the cone; the area of the mouth gape was calculated as the area of a circle and represented the largest ingestable particle. Additionally, we developed relationships between carapace length and maximum carapace width of several taxa of crustaceous zooplankton (using guidelines from Balcer et al. 1984). Lengths of ingested copepods and cladocerans were compared using analysis of variance and Tukey's multiple comparison test ($\alpha = 0.05$ unless otherwise noted).

Results

Selectivity

All sizes of walleye avoided rotifers in the laboratory ($\alpha < 0.094$; t test $P = 0.001$) and in field enclosures ($\alpha < 0.017$; $P = 0.003$). Consequently, we eliminated rotifers from all subsequent calculations of coefficients of selectivity. There were no consistent effects of prey density on selective feeding. In the laboratory, only the selectivity of small larvae feeding on medium cladocerans (0.4–0.9 mm) differed with prey density ($F_{5,8} = 5.43$, $P = 0.02$). However, regression analysis showed no linear relationship ($R^2 = 0.03$, $P = 0.53$). Selectivity by all other fish sizes on all other prey types was unaffected by prey density ($P > 0.05$). In field enclosures, the only potentially important relationship was a positive correlation for large larvae between prey density and selection for large copepods ($R = 0.66$). Although density did not consistently affect selectivity across prey taxa and size categories it did impact the ability of larval walleye to feed. In the laboratory, the number of small ($R^2 = 0.42$, $P = 0.001$) and large ($R^2 = 0.30$ $P = 0.01$) larvae with empty guts decreased with increasing prey density. All early juveniles fed, except at the lowest prey density.

We tested each prey category for differences from random feeding (reciprocal of the number of prey types (1/6); Chesson 1983). In the laboratory, prey size and taxa were important to small larvae as they selected only medium cladocerans. Small larvae avoided large cladocerans, small copepods, and large copepods. All other prey were consumed in proportion to availability (Figs. 1A and 1B). Although the pattern of selectivity was similar for small larvae in field enclosures, selectivity was not significantly different than random for any prey category (Figs. 1C and 1D). Large larvae selected against small cladocerans and small copepods in both the laboratory and field enclosures (Fig. 1). Additionally, large larvae selected for medium cladocerans and against large copepods in the laboratory. Early juveniles appeared to select prey based primarily on size, avoiding small prey of both taxa in both experimental systems and consuming other prey in proportion to availability (Fig. 1).

Growth and survival

For small larvae, prey taxa affected survival but did not strongly influence growth (Fig. 2). Small larvae that were fed cladocerans experienced greater survival than those that were fed copepods, and those larvae fed rotifers were intermediate. Small larvae that were fed high-density mixed taxa prey experienced low survival, similar to that of fish that were fed copepods ($F_{4,10} = 5.47$, $P = 0.014$; Fig. 2). Survival rates of small

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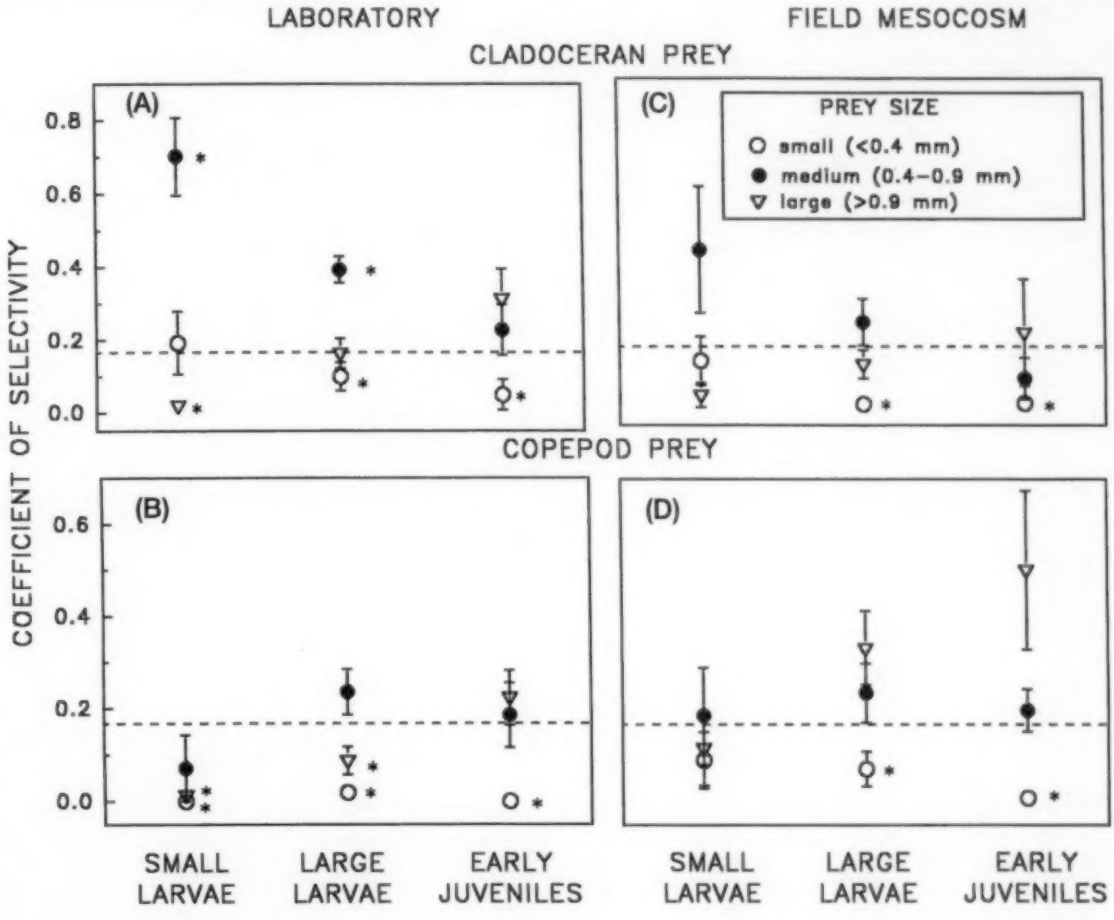
Fig. 1. Coefficients of selectivity (α; Chesson 1983; mean ± SE) by three walleye size-classes on two types of prey from laboratory experiments in 30-L aquaria (A and B) and field enclosure experiments (C and D). Broken line indicates random feeding. Prey were categorized according to size (small, <0.4 mm; medium, 0.4–0.9 mm; large, >0.9 mm) and by taxon (cladoceran or copepod). All prey density levels were combined for these analyses. Asterisks indicate values significantly different from random feeding (*t* test, with Bonferonni adjustment to insure overall error rate of 0.05).



larvae fed low-density mixed taxa did not differ from either cladoceran- or copepod-fed fish. In contrast, prey type did not affect the size achieved by small larvae at the end of the experiment (Fig. 2).

Growth and survival of large larvae were both significantly affected by prey taxa. Large larvae were the only size-class to show differences in growth with prey taxon; those fed high-density mixed taxa prey grew more rapidly than fish fed cladocerans alone ($F_{3,8} = 4.56$, $P = 0.038$; Fig. 3A). Large larvae fed cladocerans experienced higher survival rates than those fed other taxa (Fig. 3; $F_{3,8} = 53.36$, $P = 0.0001$). Survival of large larvae fed both mixed taxa prey densities was intermediate to that of fish fed either cladoceran or copepod prey ($F_{3,8} = 53.36$, $P = 0.0001$). We also assessed the effect of prey size on growth and survival of large larvae using rotifers, and small and large copepod and cladoceran prey. Growth of large larvae did not differ among either size-class of copepod or cladoceran prey. However, growth of those larvae fed rotifers, the smallest prey type tested, was somewhat lower than growth of larvae fed small copepod prey ($F_{4,10} = 3.02$, $P = 0.07$; Fig. 3C). There were no differences in survival among large larvae fed any prey size or taxa category (Fig. 3D).

In contrast to the other two walleye size groups, early juveniles experienced no differences in growth or survival with any prey type tested (Figs. 2C and 2D). Early juvenile fish growth and survival did not differ significantly when fed copepods, cladocerans, rotifers and nauplii, or mixed taxa prey of either density. Survival was lower for those fish fed rotifer and nauplii prey, but the difference was marginally insignificant ($F_{4,10} = 3.02$, $P = 0.07$).

Foraging behavior and morphometric comparisons

We measured the number of orientations, strikes, and captures of large larvae and early juveniles consuming two size-classes of copepod and cladoceran prey during 30-min experiments. Large larvae oriented (21.7 ± 5.85; mean ± SE) towards prey

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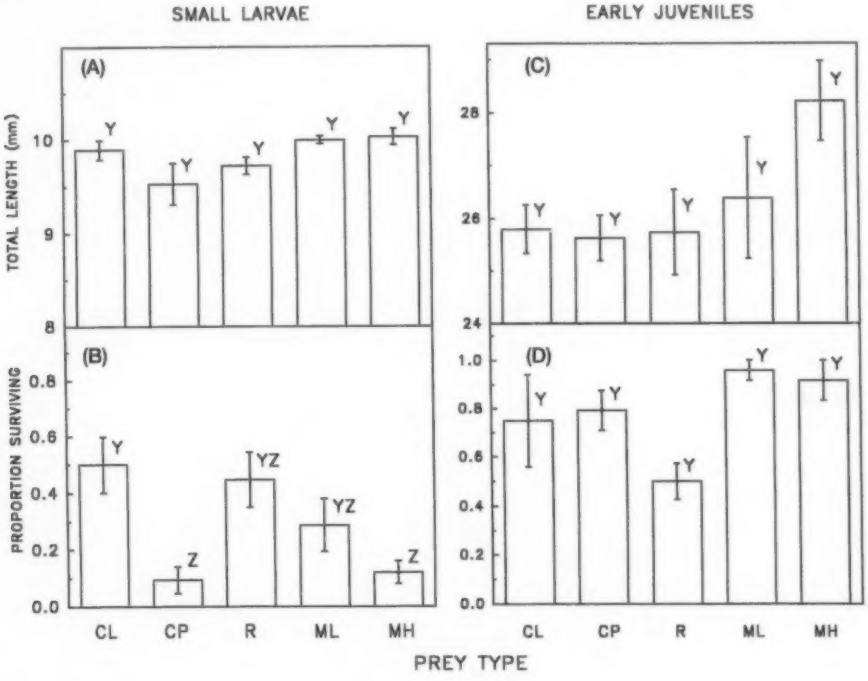
Fig. 2. Total length (A) and survival (B) of small walleye larvae after a 5-day laboratory experiment, and total length (C) and survival (D) of early juvenile walleye after a 7-day laboratory experiment feeding on different prey types (CL, cladoceran (50·L^{-1}); CP, copepod (50·L^{-1}); R, rotifer and nauplii (>500·L^{-1}); ML, mixed taxa-low density (50·L^{-1}); MH, mixed taxa–high density (100·L^{-1})). Means are of three replicate aquaria per treatment. Error bars are ±1 SE. Values with the same letters are not significantly different within each panel (MANOVA; Tukey's multiple comparison $P < 0.05$).



more frequently than early juveniles (10.94 ± 0.62; $F_{1,44}$ = 7.13, P = 0.01; Fig. 4). Both size-classes of fish oriented towards large prey more frequently than small prey ($F_{1,44}$ = 7.55, P = 0.009). The effect of fish size on the number of orientations interacted with the effect of prey taxa ($F_{1,44}$ = 4.03, P = 0.05) and the effect of prey size ($F_{1,44}$ = 6.56, P = 0.01); these effects were largely influenced by the high number of orientations towards large cladoceran prey by large larvae. Capture efficiencies (captures/strike) were lower for large larvae (0.60 ± 0.07) than for early juveniles (0.83 ± 0.03; $F_{1,44}$ = 11.71, P = 0.001). The effect of fish size on capture efficiency interacted significantly with the effect of prey size ($F_{1,44}$ = 6.21, P = 0.02) and prey taxa ($F_{1,44}$ = 5.27, P = 0.03). Also, prey size significantly affected capture efficiency with small prey being captured more efficiently than large prey ($F_{1,44}$ = 4.75, P = 0.03). Additionally, the effects of all three factors interacted significantly ($F_{1,44}$ = 7.40, P = 0.009). As with orientations, the interactions were due largely to the low efficiency of large larvae in capturing large cladocerans. Neither handling time nor reactive distance differed detectably with fish size, prey size, or prey taxa (P > 0.05). Also, there were no differences in strike distance based on prey size or taxa. However, early juveniles had significantly longer strike distances than did large larvae across all prey types combined ($F_{1,10}$ = 7.20, P = 0.02).

We compared the gape range for small and large larvae and for early juveniles with the mean and range of length of copepod and cladoceran prey consumed (Fig. 5). The mean length of cladoceran prey consumed was less than that of copepod prey for all fish sizes ($F_{1,106}$ = 18.11, P = 0.001). Maximum length of copepod prey exceeded gape in several instances; therefore, prey length may not be the best predictor of selectivity. To investigate this further we developed regression equations to relate length (L) and width (W) (mm) of *Daphnia pulex* (W = 0.6548L – 0.0709) and calanoid copepods (W = 0.2818L – 0.012 36). These equations exemplify the differences in length to width ratios between copepods and cladocerans and were used to estimate the widths of all copepods and cladocerans, respectively. The widths of all copepods were smaller than the gapes of even the smallest walleye. However, cladoceran widths could exceed the gapes of fish with small mouths.

Discussion

Our results demonstrate that young walleye fed selectively and that, for at least some size-classes, the selected prey conferred benefits in terms of greater growth or survival. Also, the relative importance of prey taxon and size to selectivity, growth, and survival changed as fish grew. Small and large larvae selected prey based on taxon and size, whereas early juveniles selected prey based primarily on size. The smallest prey types, rotifers and copepod nauplii, were uniformly avoided by all size-classes of walleye. These items occur in low numbers in young walleye diets (Bulkley et al. 1976; Mathias and Li 1982) and are probably not preferred by any size of young walleye. Our growth and survival experiments also suggested that rotifers

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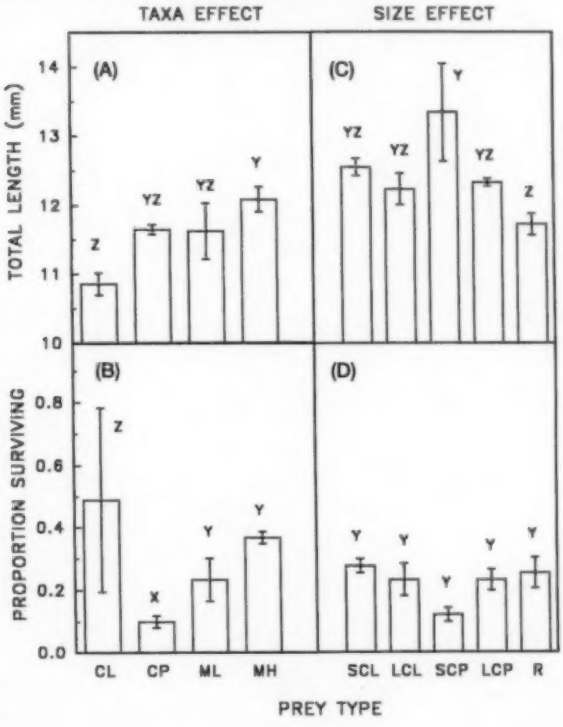
Fig. 3. Total length and survival of large walleye larvae in a 5-day laboratory experiment examining the effect of prey types (A and B) and prey sizes (C and D). In the first experiment, prey types were CL, cladoceran (50·L^{-1}); CP, copepod (50·L^{-1}); ML, mixed taxa, low density (50·L^{-1}); MH, mixed taxa, high density (100·L^{-1}); rotifer prey were not included in this experiment. In the second experiment, prey were SCL, small cladoceran (50·L^{-1}); SCP, small copepod (50·L^{-1}); LCL, large cladoceran (50·L^{-1}); LCP, large copepod(50·L^{-1}); and R, rotifer (<500·L^{-1}). Means are three replicate aquaria per treatment. Error bars are ±1 SE. Values with the same letters are not significantly different within each panel (MANOVA; Tukey's multiple comparison $P < 0.05$).



and nauplii were not profitable prey, especially to large larvae and early juveniles, but these differences were not statistically significant. We found that larval walleye selected medium-sized cladoceran prey over other prey taxa and sizes, whereas early juveniles did not. Larval fish also experienced greater survival when feeding on cladoceran prey than copepod prey, although for small larvae there was no difference between fish fed cladoceran and rotifer–nauplii prey. Both small and large larval walleye selected strongly for some size-classes of cladoceran prey and experienced greater survival when consuming cladocerans, providing good evidence that prey preference and greater profit coincide.

Increasing prey density may increase selectivity for a preferred prey size (Werner and Hall 1974; Bartell 1982) or taxon (Confer et al. 1990). In our study, neither larval nor juvenile walleye selectivity was consistently affected by prey density. Mills et al. (1986) found that prey size selectivity by young yellow perch (*Perca flavescens*) did not change with density if light levels were above 230 lx, similar to light levels used in this study. Also, prey density did not affect prey size selection in young gizzard shad (*Dorosoma cepedianum*) or bluegill (*Lepomis macrochirus*) (Bremigan and Stein 1994). However, in small larval walleye (<11 mm), prey abundance may reduce selection for larger prey items (Johnston and Mathias 1994), although this result was not consistent in all circumstances.

Mixed taxa prey at two densities allowed us to assess the effects of prey density and taxa composition. High densities of mixed taxa prey did not consistently cause significant increases in growth or survival relative to other prey treatments. A variety of indirect interactions are possible between two prey species and a single predator (Abrams 1987); for larval fish the interaction of a nonpreferred prey (copepods) with preferred prey (cladocerans) may outweigh the potential benefits of overall higher prey density. Mixed taxa treatments indicated that the presence of copepods, especially at high densities, was detrimental to small larval walleye. Perhaps copepods divert attention from the more profitable prey or behaviorally interact with the cladocerans. Extremely high densities (>500·L^{-1}) of cyclopoid copepods have resulted in larval fish mortality (Hokanson and Lien 1986). Copepods in our study were mixed taxa (calanoids and cyclopoids) and densities were substantially lower. Large larvae survived equally well with high densities of mixed taxa prey as with other prey, indicating that, although copepods were not as profitable a prey item as cladocerans, their presence did not interfere with cladoceran consumption. In contrast, young yellow perch grew faster on a copepod diet than a mixed lake assemblage or cladoceran prey (Confer and Lake 1987). Also, young bluegill and largemouth bass (*Micropterus salmoides*) growth was positively correlated to copepod presence (Lemly and Dimmick 1982). However, other studies have also found growth of young fish to be dependent on the availability of cladoceran prey (Mills et al. 1989*b*; Adeyemo et al. 1994).

All size-classes of fish consumed longer copepods than cladocerans, even larval fish that selectively consumed intermediate-sized cladocerans. In this case they should select for copepods as this would lead to greater energy returns, since the biomass of cladocerans and copepods of the same length is similar (Dumont et al. 1975) and copepods have slightly higher caloric density (Cummins and Wuycheck 1971). The shape of the two prey taxa may provide an explanation for this apparent contradiction as it relates to fish vision and gape. Cladocerans have a greater width/length ratio than copepods (0.6 for *Daphnia* vs. 0.3 for calanoid copepods). Larvae oriented more frequently towards large cladoceran prey than towards large copepod prey. Morphological characteristics of zooplankton that influence their visibility have been shown to influence the likelihood of attack (Zaret and Kerfoot 1975). Morphological traits of prey, other than length (such as shape), may be especially important to larval fish, which have limited visual acuity (Hairston et al. 1982). Some adult and juvenile fish capture copepods less efficiently than cladocerans (Drenner et al. 1978; Drenner and McComas 1980; Mills et al. 1984). However, if copepods are more difficult for young fish to see, they may not strike at a copepod unless it is very close. As a result there are fewer strikes toward copepods but similar capture efficiencies with both taxa. Consumption is reduced when feeding exclusively on copepods, leading to poor growth or mortality. However, the shape of cladocerans can also limit the size of this prey that small fish can consume. In some

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Fig. 4. Mean numbers of orientations and capture efficiency (captures/strike) (±1 SE) during 30-min experiments in laboratory arenas (2 L) by large larval (A and C) and early juvenile (B and D) walleye feeding on two prey taxa separated into two sizes (SCL, small cladoceran; SCP, small copepod; LCL, large cladoceran: LCP, large copepod).

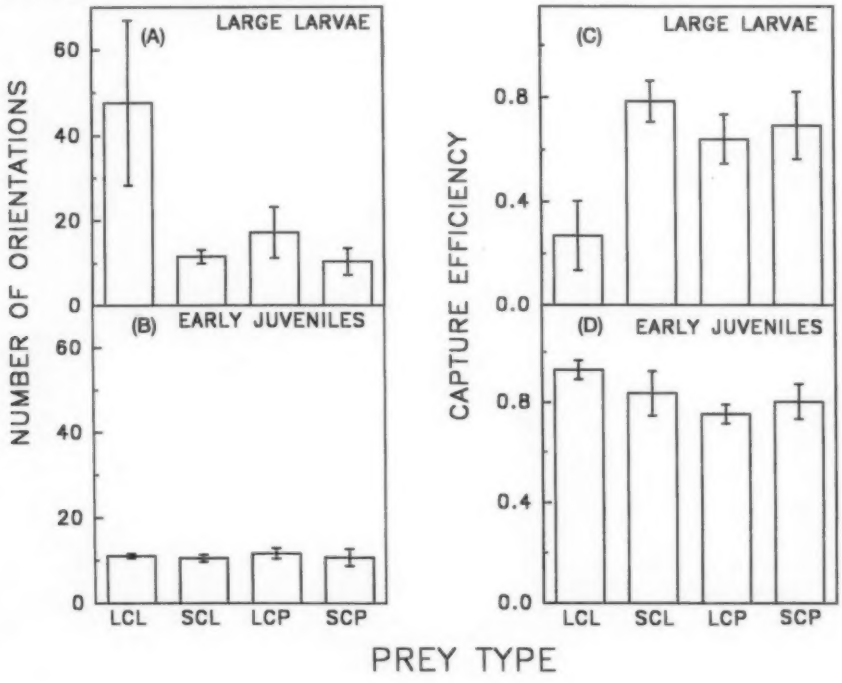
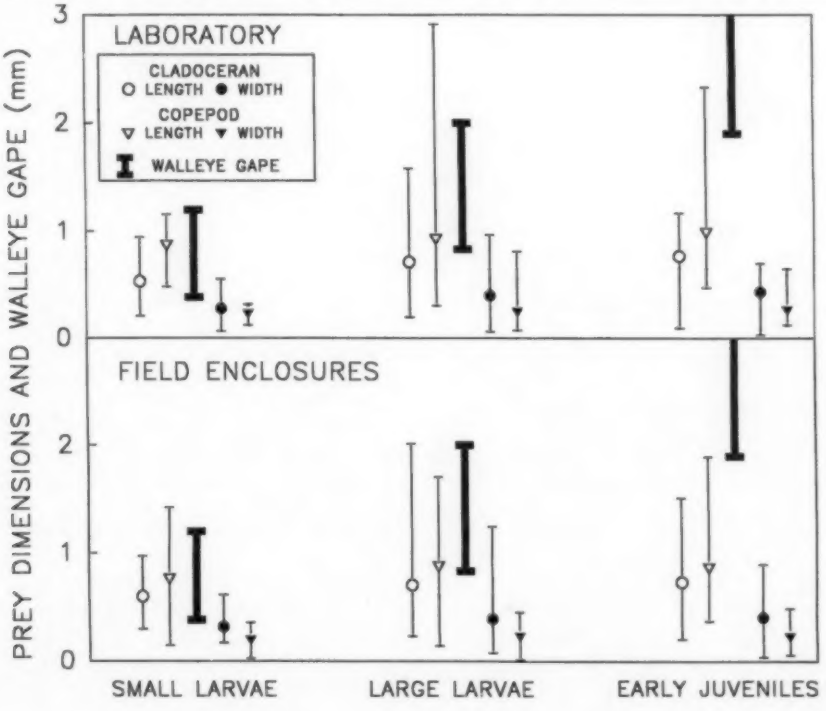


Fig. 5. Mean and range of lengths and widths of copepod and cladoceran prey consumed by three size-classes of walleye in laboratory and field enclosure experiments. Range of mouth gapes for each of three size-classes of fish were determined with a gape micrometer.



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instances, maximum length of copepods consumed exceeded fish gape indicating that prey width may be the dimension that limits consumption, as has been suggested for insectivorous birds (Sherry and McDade 1982) and for adult fish (Hambright 1991). Also, cladoceran spines, normally considered an adaptation against invertebrate predators (O'Brien et al. 1979), may impede larval fish from consuming cladocerans, especially large cladocerans. Larval walleye can find cladocerans more easily than copepods and subsequently attack and consume more of these prey, but the size of cladoceran that they can consume is limited by morphological characteristics.

We found that prey taxa and size interacted with fish size to influence selectivity and the benefits that prey conferred. These complex interactions may have caused the apparent lack of consistent patterns of prey selectivity, growth, and survival for young fish reported in the literature (Houde 1967; Merna 1977; Raisanen and Applegate 1983; Hokanson and Lien 1986; Fox 1990; Graham and Sprules 1992). Even in cases where multiple factors influencing selectivity in young fish are considered (Mills et al. 1986; Confer et al. 1990; Graham and Sprules 1992), a single zooplankton assemblage may not provide prey that span the range of important characteristics. For example, an assemblage dominated by large *Daphnia* species will not contain the small cladocerans (e.g., Graham and Sprules 1992) that were highly selected by larval walleye in this study, emphasizing the importance of presenting multiple assemblages. Prey selectivity by larval fish has important consequences for prey populations, as larval fish can significantly impact zooplankton populations (Dettmers and Stein 1992). In turn, available zooplankton prey can impact survival rates of larval fish, and this effect can be predicted by fish prey preferences.

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Defining and measuring species interactions in aquatic ecosystems

Knut L. Seip

Abstract: Based on synoptic values of observed biomasses of pairs of species in aquatic ecosystems, it is possible to distinguish types of interaction forms between the species (e.g., predation, competition, mutualism) and relate these to characteristics of their theoretical, prototype representations. For example, a distinguishing feature of observed predation was the counterclockwise rotation of time trajectories in phase space predicted by prey–predator theory. To characterize interactions, the phase portraits of the interactions were first constructed, i.e., sets of trajectories were identified by plotting biomass values for one species (or functional species group) on the *x* axis and the simultaneously observed value for the other species on the *y* axis. To characterize the phase portraits they were described by 14 "form factors," each capturing particular features of the interaction patterns. In addition seven theoretical, or prototype, interactions were characterized. Comparisons, clustering, and multivariate calibration were performed with principal component analysis and partial least squares techniques.

Résumé : À partir des valeurs synoptiques des biomasses observées de paires d'espèces dans des écosystèmes aquatiques, il est possible de distinguer des types d'interaction entre les espèces (p. ex., prédation, compétition, mutualisme) et de relier ces dernières à des caractéristiques de leur représentation théorique, prototype. Par exemple, une caractéristique distinctive de la prédation observée était la rotation antihoraire des trajectoires temporelles dans l'espace de phase prévue dans la théorie des proies-prédateurs. Pour caractériser les interactions, les portraits de phases des interactions ont d'abord été construits, c.-à-d., des ensembles de trajectoires ont été identifiés en portant sur l'axe des X les valeurs de biomasse pour une espèce (ou le groupe d'espèces fonctionnel) et, sur l'axe des Y, les valeurs observées en même temps pour les autres espèces. Pour caractériser les portraits de phases, ces derniers ont été décrits par 14 « facteurs de forme » dont chacun exprime des caractéristiques particulières des modèles d'interaction. De plus, sept interactions théoriques, ou prototypes, ont été caractérisées. Des comparaisons, la répartition en grappe et un étalonnage multivarié ont été réalisés à l'aide des techniques d'analyse des composantes principales et d'analyse de régression partielle par les moindres carrés.
[Traduit par la Rédaction]

Introduction

This work addresses the question of whether theoretical prototype interaction patterns between pairs of species, like theoretical patterns for competition, predation, and mutualism, can be recovered from observations in situ. In theory, competing species may replace each other so that an increase in the biomass of one species causes a proportional decrease in the biomass of the species being outcompeted. In phase portraits, i.e., with the biomass of the two competing species on the *x* and *y* axis, this process would be depicted as a sequence of trajectories along a line through the center point at a 135° angle to the *x* axis (cf. Gilpin et al. 1982 for assumptions underlying expected patterns for competition and mutualism). Similarly, mutualistic interactions would be depicted along a line at a 45° angle to the *x* axis. For predator–prey behavior, with the biomass of the prey depicted on the *x* axis and the biomass of the predator on the *y* axis, a characteristic counterclockwise circular rotation pattern should be found (Gilpin 1973; May 1976). However, the ideal interaction patterns between pairs of species are embedded in patterns caused by other interactions in which they partake, probably including stochasticity caused by low dimensional dynamic chaos (Ascioti et al. 1993), and in patterns caused by physical factors. My hypothesis was that, in spite of the factors disturbing the pure patterns, it should be possible to distinguish the phase portraits of prey–predator interactions from the phase portraits of competitive interactions. Examples of distinguishable pairs could be the interaction between phytoplankton, as chlorophyll *a* (chl*a*), and zooplankton and the competitive interaction between two groups of phytoplankton. Furthermore, because chl*a* is part of both the total phosphorus (TP) – chl*a* interaction and the chl*a*–zooplankton interaction, it should be possible to determine which of these cycles is closest to the theoretical prototype cycle. Thereafter, a measure of closeness could be used to assess the strength of a "bottom-up" effect (TP vs. chl*a*) relative to a "top-down" effect, (chl*a* vs. zooplankton).

To do this I first constructed phase portraits for 22 pairwise interactions where each member of the pairs was drawn among 14 biologically functional groups (including nutrients and chl*a*) monitored in Lake Mjøsa, Norway. Thereafter I characterized the phase portraits with 14 "form factors," which potentially should be able to distinguish among main interaction forms like competition, predation, and mutualism (Krebs 1995). To see how close the observed portraits would be to theoretical portraits, I also included form-factor sets for seven prototype interactions. To compare the 29 interactions (22 observed interactions and 7 synthetic interactions), I used clustering and regression techniques based on principal component analysis (PCA) and partial least squares (PLS; Martens and Næss 1989).

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Table 1. Biologically functional units observed in Lake Mjøsa, Norway, from 1986 to 1993 (data are from Kjellberg et al. 1983–1995).

Functional unit	Acronym	Unit	Mean	SD
Total phosphorus	p	$mg \cdot m^{-3}$	7.68	3.04
Total nitrogen	n	$mg \cdot m^{-3}$	460	96
Chlorophyll *a*	c	$mg \cdot m^{-3}$	2.62	1.34
Total algal biomass	a	$mg \cdot L^{-1}$	447	411
Blue-green algae	b	$mg \cdot L^{-1}$	2.59	4.71
Green algae	g	$mg \cdot L^{-1}$	4.42	4.85
Yellow algae (Crysophyceae)	y	$mg \cdot L^{-1}$	60.92	48.92
Diatoms	d	$mg \cdot L^{-1}$	280	388
Cladocera	Cl	$no. \cdot m^{-2}$	140×10^3	98×10^3
Copepoda	Co	$no. \cdot m^{-2}$	120×10^3	130×10^3
Daphnia, large	D	$no. \cdot m^{-2}$	29×10^3	46×10^3
Zooplankton (individuals)	zi	$no. \cdot m^{-2}$	380×10^3	600×10^3
Zooplankton (biomass)	z	$mg \cdot L^{-1}$	1188	945
Mysis (individuals)	m	$no. \cdot m^{-2}$	187	131

Materials and methods

Materials

The material consisted of observations from Lake Mjøsa, Norway, during the years 1986–1993. The lake is large, deep, and oligotrophic–mesotrophic. Its surface area is 335 km^2, and the average depth is 153 m (Holtan 1979; Kjellberg et al. 1983–1995). During the 8 years of observation, 14 biologically functional groups were recorded (Table 1; acronyms for each group are given in the table). With 10–12 observations per year this made for time series containing 86–91 synoptic samples. (A few samples for some groups were missing.) Since 1976 the load of total phosphorus to Lake Mjøsa has been reduced by building sewage treatment plants, by imposing restrictions on the use of agricultural land, and by prohibiting the use of phosphorus-containing detergents (Seip et al. 1987). However, during the study period 1986–1993 there has been no significant decreasing trend in either total phosphorus or phytoplankton biomass.

For eutrophic, northern latitude lakes, chl*a* shows a bimodal pattern of seasonal development (Marshall and Peters 1989). Grazing by *Daphnia* is probably responsible for the "clear-water" phase between the two algal blooms in Lake Mjøsa (see also Wu and Culver 1994). To study the interactions I paired functional groups into 22 pairs. Some of the groups were only studied tentatively and then disregarded (see Discussion). To represent the phase portraits of nutrients versus phytoplankton, I used total phosphorus vs. chl*a*. Potential competition between groups of phytoplankton was studied pairing blue-greens, greens, Crysophyceae or (hereafter) yellows, and diatoms, thereby yielding six pairs. Potential competition between zooplankton species was studied by pairing the zooplankton groups Copepoda, Cladocera, and the species *Daphnia galeata*. The reason for separating *D. galeata* is that this species, if large (>1.2 mm), may be able to graze down both the summer and the autumn phytoplankton blooms (Gliwicz 1990; Moss et al. 1991; Seip and Snipen 1993). Potential prey–predation phase patterns could be generated by phytoplankton–zooplankton interactions, thus chl*a*–zooplankton biomasses were paired to construct phase portraits. The omnivorous mysis shrimp *Mysis relicta* feeds on zooplankton and may therefore act as a fourth trophic level; therefore, I also included the phase portrait of zooplankton vs. *Mysis* (Johannsson et al. 1994).

Method

In this section, I describe the methods used to transform data from synoptic time series into statistical graphs and regressions. The sequence is as follows: construction of phase portraits, definition of form factors, and multivariate statistical treatment. The statistical results form the basis for interpretation of interaction forms between pairs of species groups.

Time series and phase portraits

Time series were first standardized according to the usual procedures of multivariate statistics (Martens and Næss 1989). Those of the time series that measured individuals per area were first log transformed to stabilize their variances. Thereafter, all time series were reduced by their respective SDs. To illustrate how features of phase portraits may capture different forms of interaction, I first discuss a phenomenological description of four interaction types (Fig. 1). The most prominent interactions are competition, predation, and mutualism (e.g., represented by separate chapters in Krebs 1995), but "facilitator–gainer" is a logical extension. (I know of no generally accepted term for facilitator–gainer.) If the time trajectory of two species is represented by two sinusoidal forms, then perfect mutualism corresponds to zero phase shift between the sinusoidal forms, and perfect competition to a phase shift of π. In prey–predator interactions the prey peaks first and phase trajectories move counterclockwise if the prey is depicted on the x axis. This corresponds to a phase shift of $3\pi/2$ between sinusoidal forms. In facilitator–gainer interactions the facilitator peaks first, phase trajectories move clockwise, and the phase shift is $\pi/2$. Thus, moving counterclockwise around a circle from zero, there are four interaction types corresponding to four patterns in the phase portraits: mutualism, facilitator–gainer, competition, and prey–predator. In aquatic ecosystems, changes in stationary states for mutualism and competition are driven by seasonal changes, e.g., temperature and light. Mutualism and facilitation are probably much less prevalent in nature than competition and predation, and mutualism, at least, has been shown to have a narrow parameter range for stable existence (May 1976, but see discussions in Wright 1989: Bertness and Callaway 1994).

Form factors

Since all phase portraits were depicted within the same frame and standardized with the same method, I simply used linear and areal measures of the phase portraits to construct the form factors. I constructed these factors so that they would express meaningful attributes of the phase portraits for predation, facilitation, competition, and mutualism. In addition, I added form factors for which I had no a priori interaction type in mind but which still would characterize the phase patterns.

Competition and mutualism

First, I calculated the least squares regression for all points representing states in phase space (e.g., all simultaneous TP_i and $chla_i$ values). The slope (v) and the coefficient of correlation (r) were used as the two first form factors. For example, if the interaction between a pair of variables was completely mutualistic, then the slope would be $v = 1.0$ and the coefficient of correlation would be $r = 1.0$ (Fig. 2, lower left panel). Secondly, the points were encircled with a curve. The ratio between the shortest and the longest axis of this closed curve (lr) was used as a third form factor. I anticipated that this ratio would correlate with r. Perfect (or "weak") competition would be captured by the same two parameters, but with $v = -1.0$.

Predation and facilitation

Since prey–predator interactions, as well as nutrient–phytoplankton interactions ideally should show a counterclockwise rotation, a coordinate system was constructed with the origin at the center of the point swarm of the phase portraits (Fig. 2, upper panel). The quadrants of the coordinate system were numbered in the usual way: quadrant I representing points with positive x and y values; quadrant II, points with negative x values and positive y values, and so on in counterclockwise numeration. The directional line (or trajectory) between

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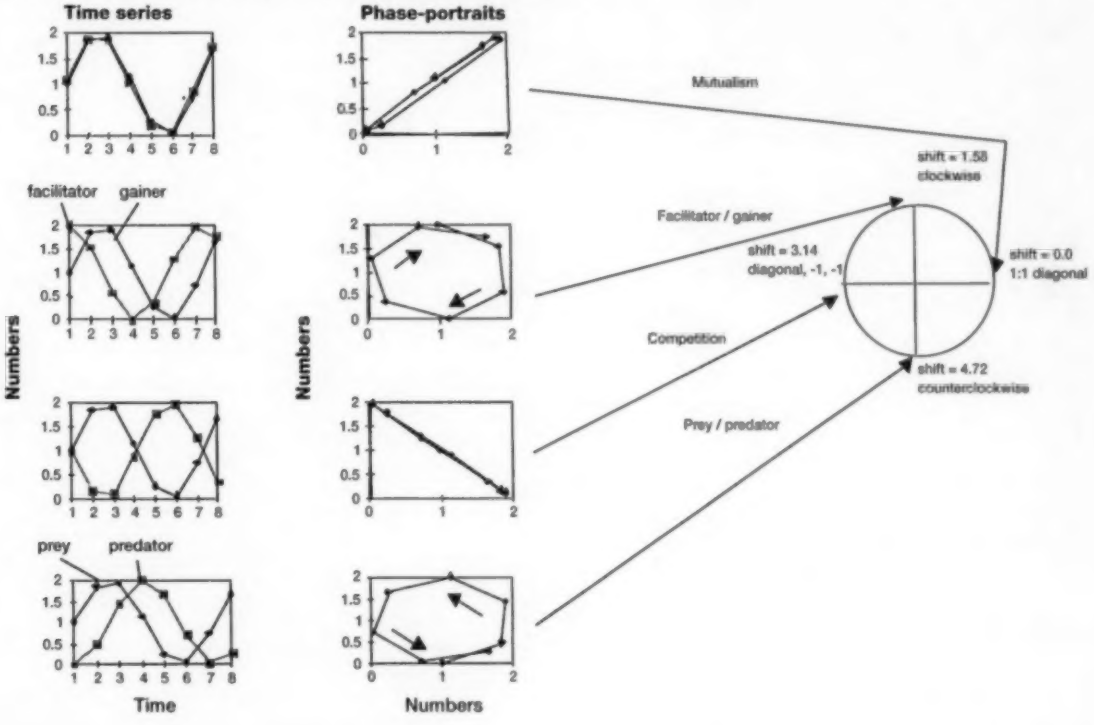
Fig. 1. Four two-species interaction forms. Time series and phase portraits for two species modeled as sinusoidal curves shifted one, two, three, and four steps of π/2 relative to one another. If the first species to peak in the time series is either the prey of, or the facilitator for, the second species, then with the first species on the *x* axis, prey–predator biomasses will rotate counterclockwise in the phase portraits and facilitator–gainer will rotate clockwise. Facilitation–gainer is suggested as an interaction form for ungulates on the Serengeti Plains of East Africa (as discussed by Krebs 1995) but rarely for open aquatic ecosystems.



successive observations at time $i-1$ and time i: (x_i-1, y_i-1) (x_i, y_i) in quadrant IV describes what happens to the biomass of the species on the *y* axis (e.g., predator) when the biomass of this species is low and the biomass of the species on the *x* axis (e.g., prey) is large. I adopted the convention that a trajectory belongs to the quadrant in which it starts. If predator biomass increases as a function of prey biomass, the angle between the trajectories and the *x* axis will be in the interval 0–90° (Fig. 2, line 2–3). Similarly, when prey biomass decreases from a small value, a large predator biomass cannot be supported, and the predator biomass will decrease. This will be expressed in quadrant II as angles for the trajectories in the interval 180–270° (e.g., Fig. 2, line 4–5). To express this feature I used two single and one compound form factors. First, I identified the phase points at the outer circumference of the point swarm and calculated the ratio (dir) of the number of points moving in counterclockwise rotations (n_{ccw}) to those moving in clockwise rotations (n_{cw}) thus $\text{dir} = n_{ccw}/n_{cw}$, where $n_{cw}^{`} = \max(n_{cw}, 1)$. In some cases I was unable to define a meaningful rotational direction because trajectories moving to and from the points were almost parallel. The form factor dir ranged between zero and infinity but was, for practical purposes, truncated at 10, i.e., 10 signifying a high proportion of counterclockwise to clockwise rotational directions. If facilitation was predominant, dir would be less than 1.0. Since the core of the cluster of points for a nonstationary prey–predator interaction would ideally be empty, the number of points within a core defined as the inner one quarter of the state space area was counted ($n_{1/4}$). The ratios (ca) of this number to the total number of points, $n_{tot} > 0$: $\text{ca} = n_{1/4}/n_{tot}$ were used as a measure of emptiness of the core space or the success of the trajectories in forming a "doughnut" shape.

To construct the first compound form factors, each quadrant was divided into two by diagonal lines cutting off the corners. The number of points (or lack of points) within each corner, c_1, c_2, c_3, and c_4 was used as a measure of "roundness." I also used the number of points within each quadrant, q_1, q_2, q_3, and q_4, as a second compound form factor to help distinguish a uniform distribution of points from a rounded prey–predator or facilitator–gainer pattern.

A general form factor

With the last form factor I tried to capture the "jaggedness" of the phase portraits. To measure jaggedness (*t*), the coordinate system centered at the midpoint of the phase portrait cluster was again used. The sum of the four distances along the *x* axis and the *y* axis from the frame to the first intersection with trajectories between the points was used as a measure of jaggedness. (The distance d_1 in Fig. 2 is one of these distances.)

Prototype interactions

I wanted to compare the phase portraits for the observed pairs of species groups (22 phase portraits were constructed based on the time series from Lake Mjøsa) to those of theoretical prototype interactions. To be able to compare the phase portraits derived from observed time series to portraits for prototype interactions, form factors for seven prototypes were constructed. Mutualism (MUT) was described as a straight line in the 1:1 direction (45°). Competition was characterized by two prototypes because observations of phase portraits for species potentially competing, e.g., two functional phytoplankton groups, showed that competitive interactions almost never occurred such that the loss of one biomass unit from a species group was replaced by an

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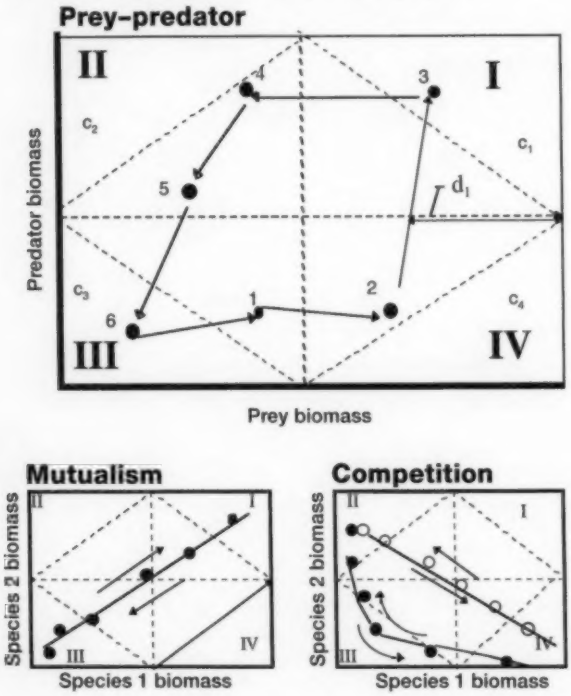

Fig. 2. Phase portrait of the interaction between two species. The biomass of species 1 is depicted on the *x* axis and the biomass of species 2 on the *y* axis. Points show simultaneously observed values for the two species. The numbers and arrows showing the sequence of observations in time. Upper panel: prey–predator interaction. The quadrants (Roman numerals), corners (outside of broken lines), and the line section d_1 are used for the definition of form factors that characterize features of the phase portrait. All time trajectories move counterclockwise in this example. Lower two panels: phase portraits for mutualism (left plot) and for weak (open circles) and strong (solid circles) competition (right plot.)

equal amount of biomass from a competing species group. (In my material, such weak competition (WCOM) was never observed. I use the term weak competition because algae may strengthen their competitive ability by discharging chemical compounds; see below.) Thus, WCOM was described by the form factors as if all points lie on a line along the diagonal forming 135° with the *x* axis and passing through the origin. "Strong" competition (SCOM) was described as if the cluster formed a boomerang shape with apex close to the lower left corner of quadrant III (low values for both species; Fig. 2, lower right panel). Such competitive patterns can arise if competition is enhanced by increasingly more optimal physical conditions for the winning species group (Emlen 1984; Seip and Reynolds 1995) or if the presence of chemical compounds associated with the competitor (but not expressed as biomass) affect the outcompeted species group (Gliwicz 1994). Furthermore, if I construct the phase portrait of the two species groups diatoms and blue-greens, one may anticipate green or yellow phytoplankton to act as intermediates (Sommer et al. 1986) and thus sharpen the bend of the boomerang.

Predation (PRE) was described as a circular form, slanted in the 1:1 direction since the prey normally get increasingly favorable ambient conditions during the season (cf., Emlen 1984; May 1976). There are few points at the corners, probably an empty central area, and counterclockwise rotation. A stochastic relationship (STO) between two species groups was described as a uniform distribution of points (Seip et al. 1990). I also included dominance by either of the species. The form factors (*Y*-D, *X*-D) were described as a narrow strip of points parallel to one of the axes. However, since the data were reduced by their respective variances, it is only possible to approach these prototypes with real data. I did not include any explicit prototype interaction for the facilitator–gainer pairs, since such pairs are rarely reported in the literature on aquatic systems (however, see Krebs 1995, p. 308, for a summary of such interactions between wildebeest and Thomson's gazelle on the Serengeti Plains; it is there also termed mutualistic behaviour).

Which features are most characteristic for predation?

It is predicted from biological theory that nutrient–chl*a* and chl*a*–zooplankton will show features of prey–predator dynamics and that algal functional groups will compete for nutrient resources (Tilman et al. 1982). To examine if, and which, form factors would reflect predation type interactions as distinct from competition type interaction, 10 observed interacting pairs were assigned a nominal value between 1 and –1 and entered in a matrix for a dependent variable, PR. (PR is represented in the first column of a 10 row × 2 column **Y** matrix. Note that the acronym PRE is used for the prey–predator prototype. Entries in the second column will be discussed below.) An interacting pair was assigned a value of 1 in the first column if it represented predation. Thus, in the subsequent multivariate analysis, form factors associated with high values in the first column of the **Y** matrix will be relevant form factors for predation. If the interacting pairs represented competition between dominant (canopy) species groups, they were given a value of –1. Competition between diatoms and blue-greens is probably weak, since diatoms usually bloom during early spring and blue-greens during late autumn in Lake Mjøsa (Kjellberg et al. 1983–1995). Competition between these two species was therefore assigned a "low" value of –0.5. It is not easy to envisage how the pattern representing a "transition form" between predation and competition should look; it might be nonexistent.

I also wanted to examine if there was any effect of the level at which the prey–predator interaction occurred, e.g., between nutrients and chl*a* (nutrients are "prey" species at trophic level 1) and between chl*a* and zooplankton (prey species at level 2). A trophic level number (TRO) was therefore assigned to the interacting pairs representing prey–predation in column 2 of the **Y** matrix (nutrients = 1, chl*a* = 2, zooplankton = 3, and *Mysis* = 4). Interactions between species at the same trophic level were assigned a value corresponding to that level.

Multivariate analysis

Multivariate statistics were used to compare and interpret patterns in phase portraits. The analysis is based on the two matrices resulting from the form-factor analysis. The first matrix, **Y** (10 × 2), contained the dependent variables predation PR and trophic level TRO. The second matrix, **X** (10–29 × 14), contained the independent form-factor variables. It is a matrix with 10–29 pairs of interactions (rows, also called objects in multivariate statistics), depending upon the analysis undertaken, and 14 form factor parameters (columns). Perfect mutualism, to give an example (see Fig. 2, lower left panel), would be coded like the following string: $s = 1.0$, $r = 1.0$, lr = 0, dir = 1.0, ca = 0.5, $c_1 = 0.25$, $c_2 = 0.0$, $c_3 = 0.25$, $c_4 = 0.0$, $q_1 = 0.5$, $q_2 = 0.0$, $q_3 = 0.5$, $q_4 = 0.0$, and $t = 4.0$. The maximum number of objects was used when I included all 22 observed phase portraits and the 7 prototypes. To analyze the patterns, PCA and PLS regression to latent structures were used because several of the form factors are correlated among themselves and therefore are not suitable for ordinary multiple regression analysis (Martens and Næss 1989; Seip 1995). The analysis was verified by cross validation on subsets chosen stochastically. The PCA and PLS methods allow several diagnostic plots, or graphs, to be constructed. The "score" plot shows points representing interacting pairs in a coordinate system formed by the principal axes defined by the PCA or the PLS algorithms (the first and the second principal axes

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Fig. 3. Diagnostic plots resulting from multivariate statistical analysis (principal component analysis). The score plot shows the clustering of interaction forms. The *x* axis shows the first principal component, which explains 23% of the variance, and the *y* axis the second principal component, which explains 21% of the variance. ClD shows the position of the interaction between Cladocera (Cl) and *Daphnia galeata* (D). Co, Copepoda; p, total phosphorus; c, chlorophyll *a*; z, zooplankton biomass; m, *Mysis*; g, green algae (Chloropyceae); b, blue-green algae (Cyanophyceae); y, yellow algae (Chrysophyceae); d, diatoms (Bacillariophyceae); PRE, prototype for predation; SCOM, prototype for strong competition (see text); WCOM, prototype for weak competition; MUT, prototype for mutualism or correlation, *Y*-D and *X*-D, prototypes for dominance by the species depicted on the *y* and *x* axis, respectively.

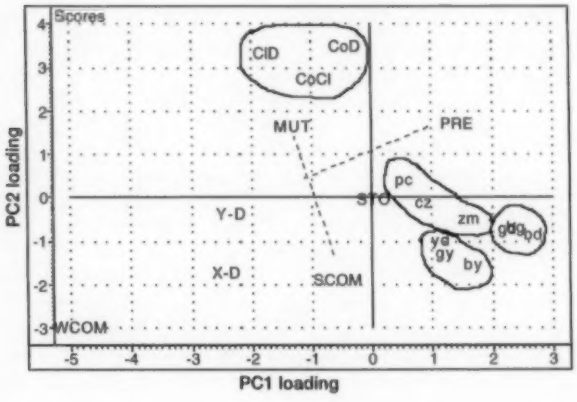
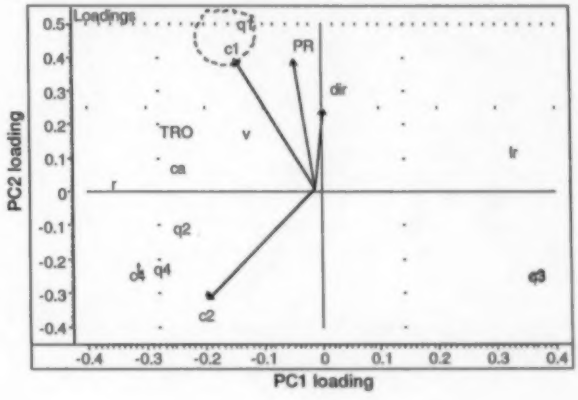


Fig. 4. The loadings show the distribution of the form factors and their relationship with theoretical predation interaction form (PR) and the trophic level (TRO; 1–4) of the prey species participating in the interaction. The *x* axis shows the first principal component, which explains 23% of the variance, and the *y* axis the second principal component, which explains 21% of the variance. *v*, regression slope for the points in the phase portraits; *r*, coefficient of variation, lr, ratio of the shortest to the longest of two orthogonal axes making an envelope for the points in phase space; ca, degree of "vacancy" in the center of the point swarm; *t*, jaggedness of the trajectory path; q_1–q_4, number of points in each quadrant; c_1–c_4, number of points in each corner (quadrants and corners are defined in Fig. 2, upper panel).



in this study). Pairs that show similar interaction patterns in terms of their form factors will be close in this graph. Furthermore, the distance from the points representing observed interactions to the points representing the prototype interactions will give information on how similar the observed pattern is to the theoretical prototype interactions. A second plot (the "loading" plot) shows the distribution of the form factors along the first and the second principal component. Form factors that can be connected to the origin with almost parallel lines (acute angles) are correlated. If the form factors lie on opposite sides of the origin, they are negatively correlated; if they are on the same side, they are positively correlated. If the lines form a right angle with each other, there is little correlation between the corresponding form factors. When the score plot (showing the pairwise interactions) and the loading plots (showing the form factors) are compared, there is an association between interactions and form factors having similar positions in the two graphs (e.g., if both lie in the upper left part of the first quadrant, the form factor in this quadrant also contributes to the characterization of the interaction form represented in this quadrant).

Results and discussion

In this section I first present diagnostic graphs (the score and the loading plots), thereafter I give an ecological interpretation of the results, and finally I show how the results can be applied in the context of the Lake Mjøsa ecosystem.

The diagnostic graphs

With a PCA, four clusters were identified in the score plot defined by the first two principal components (Fig. 3). The first and the second principal components explained 23 and 21% of the variation in the **X** data, set respectively, i.e., about an equal amount of the explained variance of 44% after two principal components. The total amount of variation explained after five principal components was about 60%. At the intersection of the two first principal components of the score plot lies the stochastic distribution (STO). To make patterns easier to see, letters representing some of the interactions have been removed (see discussion below.) The first principal component (*x* axis) has the prototype for predation PRE at its right side and mutualism MUT and both types of competition (SCOM, WCOM) at its left side. Weak competition WCOM, in the lower left corner, is far from the main cluster of points, suggesting that observed interactions similar to this prototype do not occur in the present data set. Also the prototypes *Y*-D and *X*-D were far from the main clusters. The second principal component (*y* axis) has mutualism MUT and predation PRE towards its upper end and competition (SCOM, WCOM) towards its lower end. Predation PRE is approximately at a right angle to the line connecting MUT and SCOM. The interaction pattern between zooplankton groups is clearly separate from the interaction patterns between phytoplankton groups. The zooplankton groups Copepoda and Cladocera (CoCl), being close to the prototype for mutualism, increase and decrease in concert. Replacing Cladocera as a group with *D. galeata* (CoD) does not change the mutualistic pattern to any extent. Comparison between the group of Cladocera with *D. galeata* removed and with *D. galeata* alone (ClD) also showed a mutualistic pattern.

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The patterns for phytoplankton interaction appear to occur in two distinct but close clusters. One cluster contains the three interaction pairs: blue-greens and greens (bg), blue-greens and diatoms (bd), and greens and diatoms (gd). The second cluster contains the three interaction pairs: greens and yellows (gy), yellows and diatoms (yd), and blue-greens and yellows (by). Thus in the last cluster, all are interactions with yellows.

The three predation type interactions, phosphorus–chl*a* (pc), chl*a*–zooplankton (cz), and zooplankton–*Mysis* (zm), lie in the right (predation) half plane. They are distant from MUT and at the opposite side of the origin.

The relationships between the form factors are shown in the loading plot (Fig. 4). The present loading plot is based on the **X** and **Y** matrices with 10 objects (see discussion below). As anticipated, when the ratio between the orthogonal linear dimensions of the envelope polygon, (lr) is close to one, r has a low value (i.e., angle: lr, origin, r is almost 180°). It is also seen that predation PR is closely related to counterclockwise rotation (dir), high number of points in quadrant I (q_1, c_1), low number of points in the corner of quadrant II (c_2), and low number of points in quadrant IV (q_4, c_4). Using PLS regression with cross validation (not shown), it was found that the form factors could explain almost 60% of the predation form (after six principal components, the optimal number of components determined by the PLS algorithm).

The trophic level TRO of the species group with the lowest trophic level of an interacting pair could be associated with a high v and a high r.

Ecological interpretation

The results of Fig. 3 show that it is possible to separate phase portraits of different interaction forms. Interactions between pairs of zooplankton functional groups form one cluster, and the three prey–predationlike interactions can be encircled, although they are very close to neighboring clusters. Competition between pairs of phytoplankton forms two clusters. There appears to be no competition between Cladocera and Copepoda. On the contrary, when one group increases in abundance the other group does the same. The reason may be that zooplankton groups are strongly linked to a common resource (chl*a*), which increases and decreases too rapidly to allow competitive interactions to take place (or at least to be detected with the present method). This result might have changed had the zooplankton been divided into size-classes instead of genera, but the data did not permit this.

The main distinguishing feature of predation interaction form PR was the counterclockwise rotational direction of trajectories connecting successive states of the interacting pairs (Fig. 4). There are two subsidiary distinguishing features of predation: (1) few points represent simultaneously a high prey concentration and a low predator concentration, i.e., there are few points in the fourth quadrant, and (2) few points represent simultaneously low prey concentration and high predator concentration, i.e., few points at the outer corner of the second quadrant. Similar results hold for limiting nutrient (TP) and phytoplankton (chl*a*) relationships; in that case, TP plays the role of the limiting prey and chl*a*, that of a predator. Thus, to identify a nutrient or a prey as a limiting resource, the important events are those that occur in the second and the fourth quadrants of the phase portraits (Fig. 2, upper panel).

The trophic level TRO was associated with high values for the linear regression parameters r and v. This association is probably dominated by the "mutualistic" strong correlation between zooplankton species groups at trophic level 3 and does not imply that interactions in general become more linear in phase space as trophic level increases.

The observed interaction forms are not so close to the theoretical forms as I hypothesized, but the positions are in accordance with the generic picture of relationships among the interaction forms: mutualism MUT and competition (SCOM, WCOM) are at opposite ends of the second principal component (upper and lower half planes of the score plot, respectively). The phytoplankton competition interactions are depicted on the lower half plane. They are all far from a competition form where the biomass of one species group instantaneously replaces another one, i.e., WCOM. However, in Lake Mjøsa, functional zooplankton groups seem to be forced to show a mutualistic pattern, that is, species groups tend to increase and decrease in concert. Thus, they are depicted in the upper half plane (Fig. 3). Predation PRE is roughly at right angles to a line through MUT and SCOM and positioned on the right half plane. The prey–predatorlike interactions pc, cz, and zm are all on the right half plane. The overall pattern complies with our picture of competition as a sinusoidal time series shifted π units relative to mutualism, and predation shifted $3\pi/2$ units relative to mutualism (Fig. 1, right-hand side). Using PCA techniques, the effects of forces working independently of each other, like competition and predation, tend to be projected at right angles to each other in graphical presentations: the data-driven PCA method defines new latent variables that express the maximum information content of a matrix along orthogonal vectors (PC1, PC2, etc.).

Application to lake ecosystems

Lake Mjøsa is potentially a good choice for observing interactions close to the prototype interactions I use for comparisons. The probability of finding community structures described by chainlike interactions between adjacent trophic levels, and driven by strong interactions, seems to be highest in deep, northern-latitude (>42°N) lakes (Stein et al 1995). However, Fenchel (1986) found, over some time periods, regular coupled and shifted oscillations between bacteria (prey) and flagellates (predator) in a shallow eutrophic fjord in Denmark. For Lake Mjøsa, it is generally agreed that phosphorus limits phytoplankton biomass (Holtan 1979; Kjellberg et al. 1983–1995). However, based on the score plot (Fig. 3) the interactions TP vs. chl*a* (pc) and chl*a* vs. zooplankton (cz) have no significant differences in their closeness to the predation prototype. Thus, at least for this ecosystem, it is not possible to assess the relative strength of bottom-up (TP vs. chl*a* close to predation prototype) or top-down effect (chl*a* vs. zooplankton close to the predation prototype).

To facilitate distinguishing patterns in the score plot, alternative representations of some of the interactions were represented only with dots. For example, for the phytoplankton–zooplankton interaction, chl*a* was substituted for phytoplankton biomass, and zooplankton biomass for zooplankton numbers. However, the alternative representation occupy almost the same position in the score plots as the representations they replace (full score plot not shown).

The phase patterns found are interpreted in terms of theoretical prototype interactions. However, the mutualistic pattern

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may be confounded with the pattern resulting from concerted seasonal development of two species, and competiton patterns with trajectories followed by species that are (or happen to be) π units out of phase with respect to seasonally varying abiotic forces. The last type of interaction may still be termed competition, for example, when two species compete for a common limiting resource. However, it may also be required that the interaction is mediated through a physical or metabolic link and that the size of the corresponding physical and metabolic processes are sufficient to explain the observed effect (e.g., as discussed for bacterioplankton and phytoplankton by Coveney and Wetzel 1995). Thus, the method, as it is presented here, gives suggestions of possible interaction forms between pairs of species. To firmly establish that a particular interaction has taken place, supporting evidence must be added. For aquatic ecosystems the required links for the interactions discussed here are well documented. Estimates for the magnitude of the effects, however, are not always available.

Further work

With the present data set, neither all form factor, nor all prototype, interactions gave optimum information. However, by comparing information carried by form factors, which partly express the same attributes of the phase portraits, it is possible to examine whether the factors procure the information intended. In this study, I also wanted to use simplistic, theoretical prototypes for competition, mutualism, predation, and facilitation that clearly could demonstrate how close (or how distant) observed interactions are to well-studied and well-understood interactions. However, it may be that aquatic ecosystems at northern latitudes (with very low biological activity during winter and rapid commencement during spring) only show fragments of the full cyclic patterns (cf., McCauley and Murdoch 1987). Thus, to facilitate comparisons between observed phase portraits and those of the theoretical interactions, the latter portraits should probably reflect that they are based on such fragmented time series.

Many other types of interactions can easily be envisaged, for example, switching of a predator's attention between two prey species (this would involve three species altogether).

Acknowledgments

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Effects of habitat complexity and prey abundance on the spatial and temporal distributions of perch (*Perca fluviatilis*) and pike (*Esox lucius*)

Peter Eklöv

Abstract: Structurally complex environments strongly affect the behaviours and foraging efficiencies of predators and prey. I studied temporal variation in the habitat distribution of perch (*Perca fluviatilis*) and pike (*Esox lucius*) in relation to habitat complexity and prey abundance in a lake. The study involved quantitative estimates of different habitat types, estimates of macroinvertebrate prey availability, and distribution and movement patterns of the fish. The numbers of 80–110 mm perch in the littoral zone decreased rapidly in spring, which was a result of either perch moving to the pelagic zone or predation mortality. Predation mortality is the most plausible explanation because piscivorous perch and pike >160 mm aggregated close to these high abundances of 80–110 mm perch, and 80–110 mm perch used only vegetated habitats as a possible protection against predators. Both the biomass and diversity of macroinvertebrates increased with vegetation density, whereas perch abundance was highest in an intermediate vegetation density. Pike size was inversely related to vegetation density as a result of potential cannibalism from the largest pike individuals, which preferred the tree structure habitat. Perch group size decreased with increasing vegetation density, and perch <80 mm always occurred in group sizes larger than three individuals and never occurred in the same groups as perch >160 mm. In contrast, perch >160 mm occurred at decreasing numbers with increasing group size and mainly stayed solitary or in pairs. Perch >160 mm showed no tendencies for homing behaviour and moved actively around the whole lake, whereas pike showed a strong homing behaviour. My study suggests that the structural complexity in the littoral zone can both qualitatively and quantitatively change the interaction between piscivorous predators and their prey.

Résumé : La variation temporelle touchant la distribution de la perche commune (*Perca fluviatilis*) et du grand brochet (*Esox lucius*) dans l'habitat d'un lac a suggéré que la complexité structurale de la zone littorale puisse modifier, qualitativement et quantitativement, les interactions entre les prédateurs piscivores et leurs proies. Le nombre de perches de 80–110 mm dans la zone littorale a diminué rapidement au printemps. La mortalité par prédation est une explication plus plausible de ce phénomène que la migration des perches vers la zone pélagique parce que les percidés et les brochets piscivores de taille 160 mm se rassemblaient près de ces zones de forte abondance de petites perches (80–110 mm), qui n'utilisent que les habitats couverts de végétation comme protection possible contre les prédateurs. La biomasse et la diversité de macroinvertébrés ont augmenté avec la densité de la végétation, tandis que l'abondance des perches était la plus élevée lorsque la densité de la végétation était intermédiaire. La taille du grand brochet était inversement proportionnelle à la densité de la végétation en raison de la possibilité de cannibalisme par les brochets de plus grande taille qui préféraient un habitat comportant des arbres. La taille des perches, en groupe, diminuait avec l'augmentation de la densité de la végétation et les percidés de taille < 80 mm se présentaient toujours en groupe de plus de trois individus et jamais dans les mêmes groupes que les percidés > 160 mm. Ces derniers vivaient principalement en solitaires ou en paires, ne présentaient aucune tendance en ce qui a trait au comportement de retour, et se déplaçaient activement autour du lac entier; le grand brochet présentait un comportement de retour très prononcé.
[Traduit par la Rédaction]

Introduction

Spatial heterogeneity in the environment may have large effects on the stability properties of predator–prey interactions and is an important structuring factor of communities (Murdoch and Oaten 1975; Caswell 1978; Holt 1984; Taylor 1984; McNair 1986). An increase in spatial heterogeneity may increase habitat dimensionality resulting from changes in structural complexity, habitat morphology, or other environmental variables (Schoener 1989; Warren 1989; Whitehead and Walde 1992). The dimensionality of the habitat is an important determinant of community structure because environmental dimensionality may interact with individual level variables such as the encounter rate between predators and prey (Briand and Cohen 1987; Whitehead and Walde 1992).

Structural complexity is one of those dimensions that has strong effects on the behaviours and efficiencies of predators and prey (Savino and Stein 1982, 1989; Werner et al. 1983; Eklöv and Diehl 1994; Persson and Eklöv 1995). Both the quality and quantity of structural complexity strongly affect the interaction between predators and prey as a result of

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species-specific differences in predator efficiency and prey antipredator response (Savino and Stein 1989; Angermeier 1992; Christensen and Persson 1993; Eklöv 1995). Different types of predators are differentially affected by varying degrees of structural complexity as a result of both structural complexity interacting with foraging mode and encounter frequency with prey decreasing with an increase in structural complexity (Savino and Stein 1982, 1989; Eklöv and Diehl 1994).

In lakes, habitat heterogeneity is most commonly present in the form of littoral zone vegetation or a depth-gradient diversity both of which may have profound effects on species abundance and diversity (Werner et al. 1977; Tonn and Magnusson 1982; Eadie and Keast 1984; Benson and Magnusson 1992). Greater diversity of habitats within a lake appears to create an opportunity for a more species diverse fish community (Benson and Magnusson 1992), and different forms of structural complexity may provide shelter for fish to escape predators (Werner and Hall 1988; Mittelbach 1988). Prey refuges are species specific, suggesting that the survival of prey will depend on the availability of different types of refuges in the environment (Christensen and Persson 1993). Alternatively, prey may use schooling as an antipredator strategy (Christensen and Persson 1993; Eklöv and Persson 1995). The efficiencies of piscivorous predators are affected by the degree of structural complexity: the efficiency of sit-and-wait predators increases with an initial increase in structural complexity whereas the efficiency of active group foraging predators decreases with an increase in structural complexity (Savino and Stein 1982, 1989; Anderson 1984; Eklöv and Diehl 1994). Size-dependent spatial distributions of piscivores as a function of interactions between individual predators of different sizes may also affect the efficiency of piscivorous predators (Eklöv 1992; Eklöv and Diehl 1994). For example, large piscivorous pike (*Esox lucius*) were found to interact with smaller pike and stayed closer to the prey than did smaller pike (Eklöv 1992; Eklöv and Diehl 1994).

The aim of this study was to test predictions concerning patterns of the temporal variation in habitat distribution of perch (*Perca fluviatilis*) and pike in relation to habitat availability and structural complexity in a small lake. The predictions were created from previous experimental results that revealed mechanistic explanations for the outcomes of predator–prey interactions between large and small individuals of perch and pike (Eklöv 1992; Eklöv and Diehl 1994; Diehl and Eklöv 1995; Eklöv and Persson 1995).

Perch and pike are the two most widely distributed fish species in Scandinavian lakes (Svärdson 1976). In lakes where they co-occur, perch totally dominate numerically in the pelagic zone whereas both species commonly occur in the littoral zone (Persson et al. 1991). The two species have been found to have both numerical and behavioural effects on prey fish populations (Persson et al. 1996). During their ontogenetic development, perch undergo several niche shifts with respect to food resources and habitat use (Persson 1988). With increasing body size, perch switch from being zooplanktivorous mainly in the pelagic zone to benthivorous feeding mainly in the littoral zone. If they become large enough, perch feed mainly on fish and use both the littoral and pelagic zones (Persson 1988). As piscivores, perch are efficient group foragers (Eklöv 1992), but their efficiency decreases in structurally complex environments (Eklöv and Diehl 1994; Persson and Eklöv 1995). Pike are sit-and-wait predators that occupy vegetated zones of lakes (Diana et al. 1977; Diana 1980). The foraging efficiency of pike increases at low vegetation densities and decreases at high vegetation densities (Savino and Stein 1989). As a consequence, pike are less affected by the presence of structurally complex prey refuges than piscivorous perch (Eklöv and Diehl 1994). In relation to differential predation risks and predator efficiencies in different habitats, the abundances of the smaller sizes of perch and pike were predicted to be positively related to an increase in structural complexity, whereas the abundances of larger sizes of perch and pike were predicted to be negatively related to an increase in structural complexity. The abundance of smaller sizes of perch was predicted to be positively related to an increase in abundance and diversity of macroinvertebrates. As a result of the social behaviour of perch and the territorial behaviour of pike (Eklöv 1992; Eklöv and Diehl 1994), perch could be expected to be active and form groups whereas pike could be expected to be sit-and-wait and solitary. However, because the shoaling ability of fish interact with structural complexity (Pitcher 1986), the group size of perch was predicted to be inversely related to vegetation density, and large perch were predicted to stay solitary and inactive, which has also been found for large perch with increasing structural complexity (Eklöv and Persson 1995). The abundances of large perch and pike were predicted to be positively related to the abundance of small perch.

Materials and methods

System studied

The study was performed in Lake Degersjön 25 km west of Umeå, Sweden (63°50′N, 20°1′E), during 1990–1991. All data presented were collected in 1990 except for the gillnet samples and movement patterns of perch and pike >160 mm from 1991, which were included to estimate variability of movement patterns between years. Lake Degersjön is a primarily spring-fed, low-productivity lake with a small outlet that dries out in summer. The lake is shallow (maximum depth 6 m) and has a surface area of 27.3 ha. Water transparency (Secchi depth) varies between 3.5 and 4.6 m. The lake is surrounded by a mixed spruce and pine forest except for a deforested area, which covers one third of the western shore. The littoral zone vegetation consists of the following emergent macrophytes in descending order of abundance: *Carex rostrata*, *Phragmites australis*, and *Equisetum fluviatile* (Fig. 1). Submerged and floating leaf vegetation are also present at lower abundances and consist of *Isoetes* sp., *Lobelia dortmanna*, *Nymphaea alba*, *Potamogeton natans*, and *Sparganium* sp. Along the shore, several dead trees have fallen into the lake.

Physical structure

The physical structure of the lake was quantified from aerial photographic slides that covered the whole littoral zone of the lake taken from an approximate height of 100 m in July 1990, and from observations obtained during the electrofishing (see methods for fish abundance estimates below). The littoral zone was classified into the following eight categories: *Carex rostrata*, *Equisetum fluviatile*, *Phragmites australis*, *Salix* spp., sand, tree structure, and bog margin. *Equisetum fluviatile* constitutes a similar structure type as *Carex rostrata* so their abundance were pooled. The locations of the fish were related to the habitats quantified from the photo slides. Physical structures in transects were at three locations in the lake (Fig. 1) estimated on one occasion in July 1990 by an observer, who classified vegetation into five density categories (very dense, 150 stems/m^2; dense, 60–150 stems/m^2; sparse, 40–60 stems/m^2; very sparse, 5–40 stems/m^2; and no vegetation) for every square metre of the transects. The

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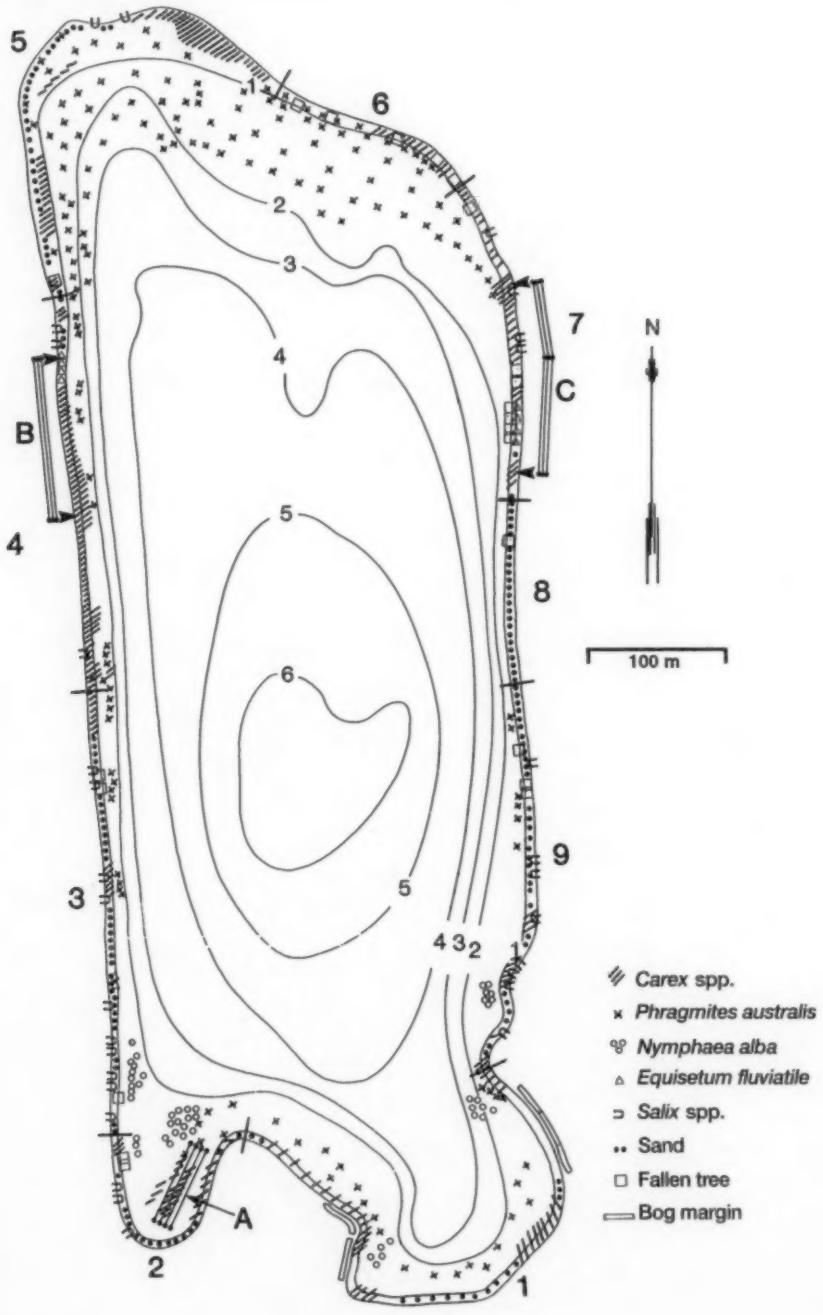
Fig. 1. Morphometric map of Lake Degersjön. The numbers 1–9 indicate the different zones and A–C indicate the three locations where underwater observations were conducted.



presence of trees fallen into the lake and water lilies (*Nymphaea alba*) was also noted. All categories of vegetation density were present at the three locations.

Fish abundance estimates

The main fish species present in the lake are perch and pike. In addition, roach (*Rutilus rutilus*) and burbot (*Lota lota*) occur in small numbers. A small population of large roach (range 240–310 mm) was also present in the lake. During the electrofishing, schools of smaller roach were only observed twice in the littoral zone (15–20 individuals of 1-year-old roach). A small number of burbot were also caught in the littoral zone. The size distributions of the fish species in the lake

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Table 1. Mean depth (cm) and range (given in parentheses) for transects at locations A, B, and C.

Transect	Location A	Location B	Location C
1	80 (62–98)	39 (37–41)	38 (25–44)
2	75 (58–91)	63 (60–65)	67 (40–80)
3	70 (57–83)	90 (86–96)	94 (43–145)
4	63 (55–70)		

were estimated in July 1991 based on gillnet samples (benthic multimesh gill nets, 56 m total length, mesh sizes: 9.5, 14.5, 18, 24, 29.5, 33, 38, and 46 mm) and expressed as numbers per four net panels. Two nets were set in the deepest part of the lake and two nets were set immediately outside the littoral zone. All nets were set in the afternoon (15:00) and lifted the following morning (09:00).

The lake was divided into nine zones (100–440 m long; Fig. 1) whose demarcations were based on physical structure and lake morphometry. Fish abundance was estimated by electrofishing, covering all zones of the lake and repeated at approximately 1-week intervals during the same time of the day (between 09:00 and 15:00) between May and September. This was done both in 1990 and 1991. Data is expressed as catch per unit effort (CPUE) (catch per one electrofishing round the lake). I used mark and recaptures of perch and pike >160 mm to estimate population size. The electrofishing was conducted from a row boat. The anode was dipped into the water at 3-m intervals and stunned fish were counted and classified. Perch were grouped into four size-classes (<80, 80–110, 111–130, 131–160, and >160 mm; perch <80 mm and >160 mm are hereafter called small and large perch, respectively) and pike into two size-classes (<160 and >160 mm; hereafter called small and large pike, respectively). Fish from the largest size-class were placed in a tub with lake water for later tagging. The number of dips of the anode into the water was counted for each zone to be able to keep track of the location of the captured fish. When a zone had been fished, all collected large perch and pike were anaestesized with ethylene glycol monophenylether and individually tagged with floy tags (Floy Tag & Manufacturing, Seattle, Wash.). Fish were allowed to recover in a tub containing lake water before they were released back into the lake. Because large perch and pike were released at the boundary between two zones after the tagging procedure, they had the possibility to choose between going back to the zone in which they had been caught, going to the next zone (neighboring zone), or going to one of the other zones. Given these options, the homing behaviour and movements within and among zones of later recaptured large perch and pike could be estimated. This was done by noting the locations of recaptured tagged large perch and pike at the next and later electrofishing samplings.

Underwater methods

Underwater estimations of fish abundance in relation to habitat type were performed using a visual census strip count technique (Keast and Harker 1977; Turner and Mackay 1985; Helfman 1983). At three locations in the lake (A, B, and C), transects parallel to the shore were constructed using 60–137 m long nylon cords (Fig. 1). The four transects at location A were placed to cover the macrophyte vegetation in the small bay at the southern end of the lake. The three transects at each of the other two locations were placed 3 m from the shore separated by 3 m extending 9 m out in the lake. The transects at location A were numbered starting with the most eastern transect as number one. The transects at locations B and C were numbered starting with the transect closest to the shore as number one. The water depth was measured at the ends and at the center of the transects (Table 1). Different colored plastic bands were attached to each cord, dividing them into 1-, 5-, and 10-m intervals. The cords were about 20 cm from the bottom and the end of the cords were attached to wooden poles that were buried into the sediment.

All transects were visually censused using face mask and snorkel on eight occasions during July and August in both 1990 and 1991. The surveys were conducted between 10:00 and 15:00 using the same census method. At all observations, the water was clear enough to allow an observation distance of at least 4 m. Location censused was selected at random. At the chosen location, the observer swam down to the center of transect number one recording all observed fish within a 3 m wide strip. For every meter the fish were classified by species and size using the same classification norms as for the electrofishing samplings. In addition, perch were classified into four group sizes (1, 2, 3–10, >10 individuals). Because perch groups consisted of different-sized perch individuals, the numbers of perch of each size-class were estimated for each group size. The second transect selected was number three and when this transect had been covered, the observer changed to another location. After these two transects in each location had been surveyed, the same procedure was repeated for the remaining transects. This procedure was used to minimize the risk that potential disturbance of fish when surveying the other transects biased observations of the middle transect. Before the survey started, the observer was trained in the observation procedure to minimize disturbance of the fish, as perch and pike reacted differently to the observer. A constant swimming speed (approximately 10–15 m/min) of the observer was of major importance when encountering perch because perch tended to aggregate around an immobile observer. In contrast, when encountering pike it was important that the observer moved very slowly (<10 m/min) not to frighten the pike over to another transect that had not yet been surveyed. If the observer moved slowly, the pike retreated, swam slowly around the observer, and ended up at the same location as when encountered by the observer. This method has been shown to be successful for estimating population sizes of pike (Turner and Mackay 1985).

Macroinvertebrates

Macroinvertebrates were sampled in July 1990 at eight sites each in locations B and C. Three sites were chosen at each vegetation density except for the highest vegetation density, where four sites were chosen. At each site, six samples were taken with a Plexiglas core (area 38 cm^2) and the samples were sieved through a 0.5-mm net, preserved in 70% ethanol and stained with rose Bengal. In the laboratory, the animals were classified by taxa, measured, and counted. Lengths were converted to dry masses using length–mass regressions (Persson and Greenberg 1990).

Statistical methods

All relationships between fish abundances or group size and depth or vegetation density were tested by linear or quadratic regressions. Diversity of littoral macroinvertebrates was estimated by Shannon–Weaver index. Large perch, small pike, and large pike selectivities for high density of perch (80–110 mm) were tested with Mann–Whitney *U* test based on estimates from Ivlev's electivity index. All proportions were tested with the *G* test and all other statistical analyses were done using ANOVAs. Data were $\ln(x)$ or $\ln(x + 1)$ transformed when necessary to stabilize variances.

Results

Littoral and benthic fish abundance

Based on gillnet and electrofishing data combined, perch were the numerically dominant fish species in the lake, and the population size structure showed a distinct year-class of 2+ perch ranging from 85 to 110 mm (Fig. 2). Larger perch consisted of a wide distribution of perch sizes >120 mm. All pike were caught by electrofishing except for one individual caught in the littoral net.

Perch between 80 and 130 mm were abundant in the littoral zone in May, after which they rapidly decreased to about one

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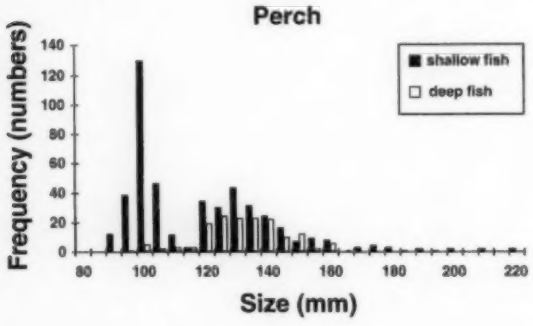
Fig. 2. Size–frequency distribution of perch caught close to the littoral zone (shallow fish) and in the deepest part (deep fish) of the lake in 1990.



quarter of the level in May (Fig. 3). The numbers of small and large pike captured in the littoral zone were similar and did not depend on whether the high abundance of small pike in September was included in the analyses (September observation included $t = -0.208$, df = 8, $p = 0.841$, September observation excluded, $t = -1.389$, df = 7, $p = 0.207$).

Littoral population sizes of large perch and pike were estimated to be 114 ± 43 (mean ± 95% CL) and 205 ± 95 individuals, respectively, in 1990 and 164 ± 93 and 140 ± 62 individuals, respectively, in 1991. None of the tagged large perch or pike were captured in the gill nets, suggesting that movement between the littoral and pelagic zones was limited.

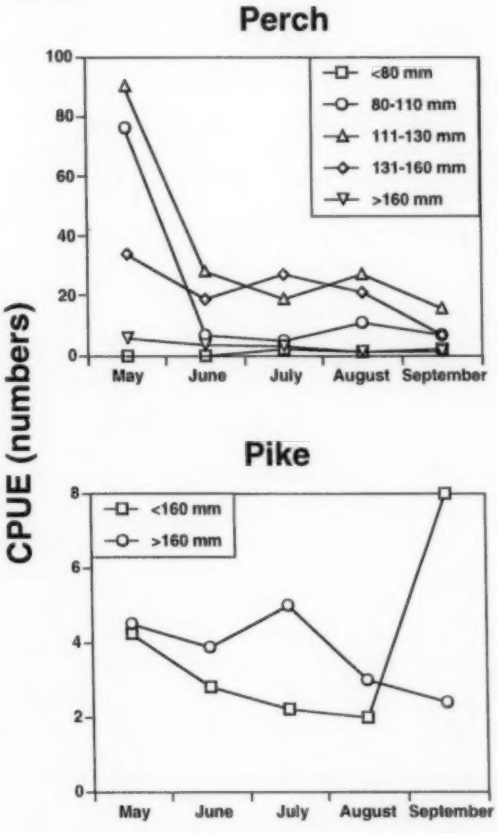
Littoral resources

The biomass and diversity of littoral macroinvertebrates did not differ between locations B and C (biomass, $t = 1.149$, df = 14, $p = 0.270$; diversity, $t = -0.129$, df = 14, $p = 0.899$); hence, the data from the two locations were pooled (Fig. 4). The biomass and diversity of littoral macroinvertebrates increased with vegetation density (one-way ANOVA on biomass, $F_{4,11} = 4.587$, $p = 0.023$; on diversity index, $F_{4,11} = 3.920$, $p = 0.036$). The tendency for higher biomasses in the zero vegetation samples was a result of the high biomasses of Oligochaeta and Chironomidae in these samples, the dominance of which was reflected in low diversity.

Perch and pike habitat selection

All perch size-classes, except the smallest, differed in habitat use from what could be expected if they were using habitats randomly (Fig. 5, Table 2). The lack of a significant deviation from a random use for the smallest size-class of perch was probably due to the low sample size. The significant difference between habitat use of perch 80–160 mm and random habitat use was mainly a result of a higher proportional use of the *Carex* habitat and a lower proportional use of the sand habitat (Fig. 5, Table 2). Large perch used a higher proportion of mainly tree structure and *Salix* habitats compared with random habitat use (Fig. 5, Table 2). Perch ranging from 80 to 110 mm and large perch changed habitat over time (Fig. 5, Table 2). The only habitat preferred by perch between 80 and 110 mm in July was the *Carex* habitat, whereas they increased the use of the *Salix* habitat in September (Fig. 5, Table 2). Overall, large perch used a higher proportion of the tree structure and

Fig. 3. Numbers (CPUE) (catch per electrofishing round) of perch and pike of different sizes caught by electrofishing from May to September 1990.



Salix habitats. They increased their use of the tree structure habitat from May to July at the same time as they decreased their use of the *Carex* habitat. In contrast, large perch decreased the use of tree structure from July to September and increased their use of *Carex* and *Salix* habitats. The fact that more than 60% of the habitats used during July and August consisted of bog margin, tree structure, and *Salix* demonstrate that large perch selected more open habitats during these months.

Both size-classes of pike differed in habitat use from what could be expected if they were using habitats randomly (Fig. 5, Table 2). For both small and large pike, this was mainly due to their preference for the *Salix* habitat and avoidance of the sand habitat (Fig. 5, Table 2). Small pike did not use *Phragmites* habitats at all, and their habitat use did not change over time (Table 2). Large pike used the *Salix* habitat heavily in May and increased their use of the tree structure habitat from May to July. The use of tree structure decreased in August and the use of *Salix* increased during September (Fig. 5, Table 2). The high use of bog margin, tree structure, and *Salix* habitats in July suggest that large pike preferred more open habitats during this month. Large pike that were found in tree structure were also the largest pike found in the lake.

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Fig. 4. Biomass (mg/m^2) and diversity (Shannon–Weaver index) of littoral macroinvertebrates in location B and C 1990. Values are means ± SEs of three replicates per vegetation density level, except at the highest vegetation density where four replicates were measured.

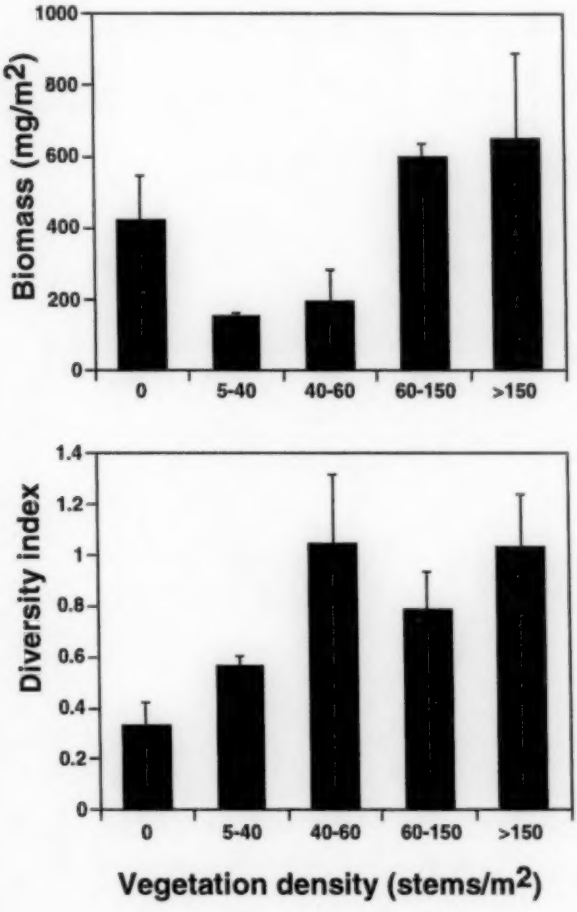
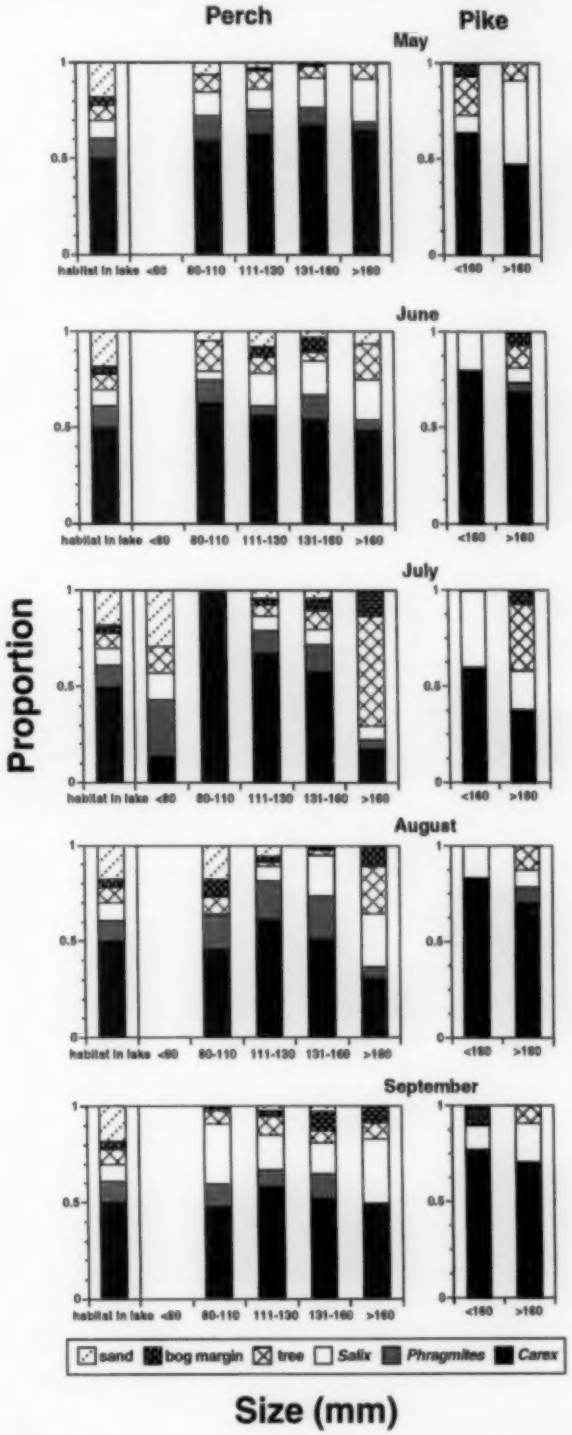


Fig. 5. Proportional habitat use of the different sizes of perch and pike in May–September 1990. The leftmost bar in the perch panels shows the proportions of the different habitats available in the lake.



Perch abundance, vegetation density, and depth

Because of the small difference in depth among transects at location A, no analysis of the variation of vegetation density and perch abundance with depth was performed for this location. The vegetation density at location B decreased with increasing water depth (Fig. 6; $R^2 = 0.529$, $F_{1,22} = 24.69$, $p < 0.0001$), whereas no relationship between vegetation density and water depth was present at location C ($R^2 = 0.004$, $F_{1,38} = 0.168$, $p = 0.684$) because the vegetation was more sparse in the shallow areas of location C than at the other locations.

Perch abundance increased with depth at location B ($R^2 = 0.529$, $F_{1,22} = 24.69$, $p < 0.0001$) but was unrelated to depth at location C (Fig. 6; $R^2 = 0.004$, $F_{1,38} = 0.168$, $p = 0.684$). Perch abundance first increased with vegetation density then decreased at high vegetation density (test of difference between a linear and a second-degree polynomial: location A, $F_{2,37} = 28.61$, $p < 0.0001$; location B, $F_{2,37} = 53.66$, $p < 0.0001$; location C, $F_{2,37} = 158.11$, $p < 0.0001$; regression on second-degree

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Table 2. *G* tests of the proportions of different habitats used by five size-classes of perch and two size-classes of pike compared with the proportions of the habitats available in the lake.

	df	*G*	*p*
Perch H_0 habitat[a]			
<80 mm	5	3.16	0.532
81–110 mm	5	43.48	<0.0001
111–130 mm	5	97.46	<0.0001
130–160 mm	5	94.27	<0.0001
>160 mm	5	91.46	<0.0001
Perch H_0 time[b]			
81–110 mm	5	11.88	0.036
111–130 mm	5	0.71	0.983
130–160 mm	5	1.72	0.886
>160 mm	5	12.35	0.030
Pike H_0 habitat[a]			
<160 mm	5	29.58	<0.0001
>160 mm	5	53.79	0.0008
Pike H_0 time[b]			
<160 mm	3	1.99	0.57
>160 mm	3	8.10	0.044

[a]H_0 habitat, proportion of habitat used by perch and pike did not differ from the proportions of habitat present in the lake.

[b]H_0 time, the proportion of habitat used by perch and pike did not vary with time.

polynomial (Fig. 6): location A, $R^2 = 0.436$, $F_{2,37} = 14.298$, $p < 0.0001$; location B, $R^2 = 0.592$, $F_{2,37} = 26.832$, $p < 0.0001$; location C, $R^2 = 0.810$, $F_{2,37} = 79.096$, $p < 0.0001$).

The size distribution of pike and vegetation density

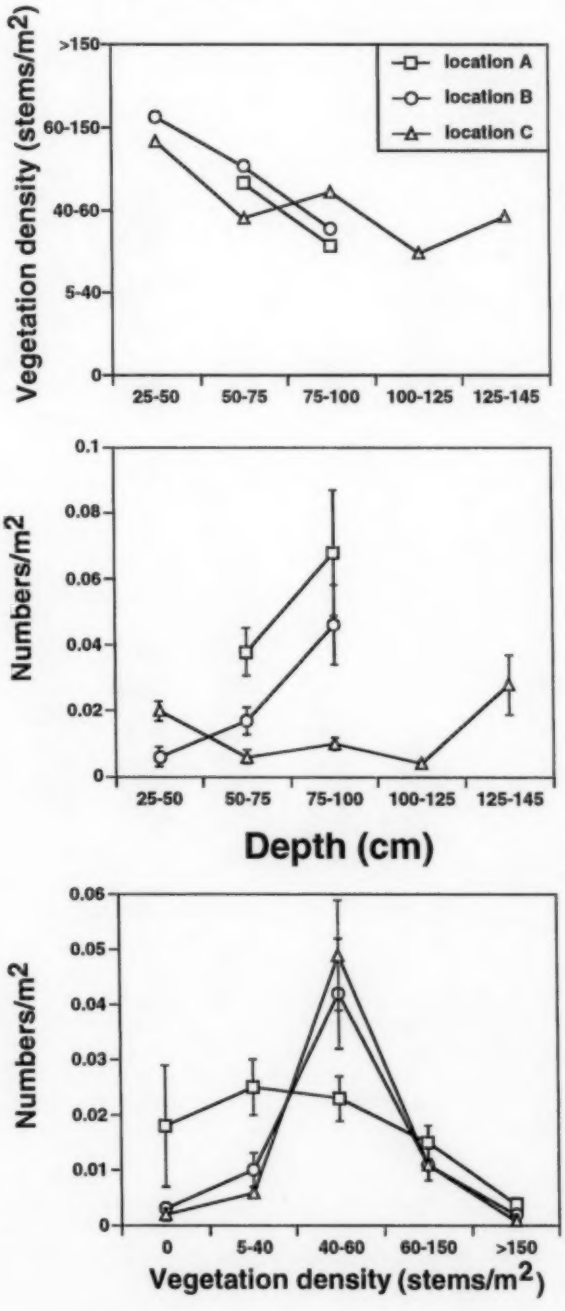
The size of pike was inversely related to vegetation density at all three locations (Fig. 7; location A, $R^2 = 0.44$, $F_{1,25} = 19.618$, $p < 0.0001$; location B, $R^2 = 0.302$, $F_{1,35} = 15.125$, $p < 0.0001$; location C, $R^2 = 0.307$, $F_{1,20} = 8.873$, $p = 0.007$).

Perch group formation and vegetation density

The group size of perch decreased with increased vegetation density at locations A and B (Fig. 8; location A, $R^2 = 0.673$, $F_{1,28} = 57.54$, $p < 0.0001$; location B, $R^2 = 0.542$, $F_{1,34} = 37.36$, $p < 0.0001$). At location C, the group size of perch first increased with vegetation density but decreased at high vegetation density (test of difference between a linear and a second-degree polynomial: $F_{2,37} = 248.73$, $p < 0.0001$; regression on second-degree polynomial (Fig. 8): $R^2 = 0.822$, $F_{2,37} = 124.52$, $p < 0.0001$). The large variation in group size in the absence of vegetation at location A was due to one observation of 120 individuals of perch 80–110 mm. Schools (>10 individuals) of perch 80–110 mm were also observed in the absence of vegetation at the other two locations. Perch in these schools were also found to forage on zooplankton in the open water.

The numbers of perch in each group size (1, 2, 3–10, and >10 individuals) differed between group sizes for all size-classes except for perch of size 111–130 mm (Fig. 9). Perch <111 mm were more frequent in larger groups than in smaller groups and perch <80 mm never occurred in the same groups as large perch (one-way ANOVA on perch <80 mm, $F_{3,8} = 9.37$, $p = 0.005$; on perch 80–110 mm, $F_{3,8} = 8.15$, $p = 0.008$). A Bonferroni-adjusted post-hoc comparison revealed that

Fig. 6. Vegetation density (stems/m^2) and perch abundance (no./m^2) versus depth (cm), and perch abundance versus vegetation density at locations A, B, and C. Values are means ± SEs for the period May–September 1990.



perch 80–110 mm were more frequent in groups of 1 or 2 individuals than 3–10 individuals (1 vs. 3–10, $p = 0.029$; 2 vs. 3–10, $p = 0.024$). Perch 111–130 mm occurred in similar numbers in the different group sizes (one-way ANOVA, $F_{3,8} = 2.32$,

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Fig. 7. Pike size (mm) as a function of vegetation density (stems/m^2) at locations A, B, and C. Values are means ± SEs for the period May–September 1990.

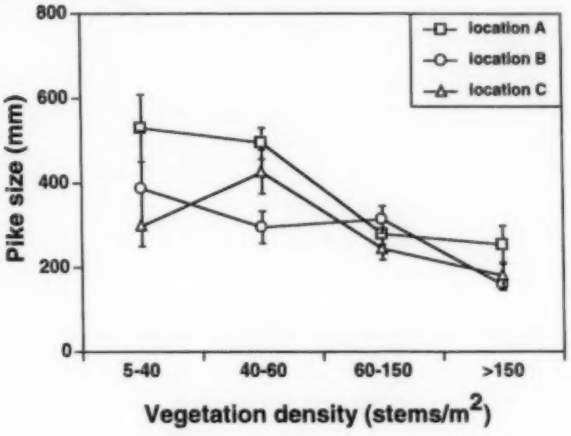
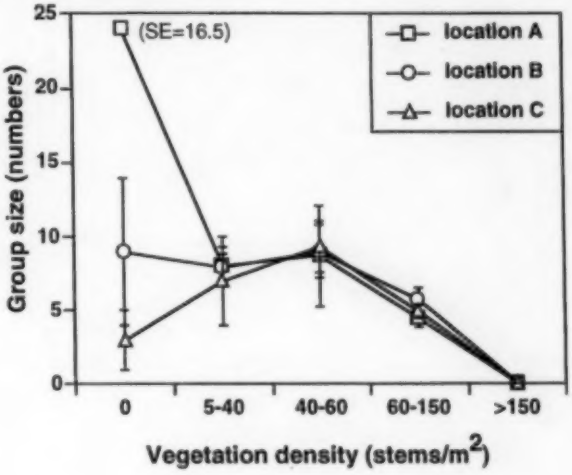


Fig. 8. Group size (nos.) of perch at the different vegetation densities at locations A, B, and C. Values are means ± SEs for the period May–September 1990.



$p = 0.152$). Perch 131–160 mm occurred most frequent in the groups of 3–10 individuals (one-way ANOVA, $F_{3,8} = 9.19$, $p = 0.006$). A Bonferroni-adjusted post-hoc comparison revealed that perch 131–160 mm were less frequent in groups of 2 individuals ($p = 0.05$) and >10 individuals ($p = 0.005$) than in groups of 3–10 individuals. Perch >160 mm decreased in numbers with increasing group size (one-way ANOVA, $F_{3,8} = 5.13$, $p = 0.029$). A Bonferroni-adjusted post-hoc comparison revealed that perch were more frequently solitary ($p = 0.034$) or in pairs ($p = 0.05$) than in groups of >10 individuals.

Movement patterns of large-sized perch and pike

Large perch showed a little tendency to stay in the same zone over time in both 1990 and 1991 (Fig. 10; $G = 1.48$, df = 2, $p = 0.478$). In contrast, pike preferred either to stay within the same zone over time or moved to the neighboring zone both in 1990 and 1991 ($G = 14.078$, df = 2, $p = 0.0009$). Thus, pike showed a stronger homing behaviour than perch, staying at higher proportions in the same or neighboring zones compared with perch ($G = 11.37$, df = 2, $p = 0.0034$). A comparison of perch and pike movements between 1990 and 1991 revealed that both species showed little tendencies to stay within the same zone (perch: $G = 1.55$, df = 2, $p = 0.460$ pike: $G = 1.95$, df = 2, $p = 0.378$).

The pike that stayed in the same zone between years were the largest pike found (size range 550–800 mm). Both large perch and pike selected the parts of the transects that had the highest densities of perch 80–110 mm (Mann–Whitney U test on Ivlev's selectivity index, both large perch and pike, $n = 3$, $p = 0.05$), whereas small pike avoided these high-density perch patches (Mann–Whitney U test on Ivlev's selectivity index, $n = 3$, $p = 0.05$). At one occasion, a pike (size 400 mm) was observed to attack and capture one perch 80–110 mm.

Discussion

Studies on species interactions have demonstrated that the degree of structural complexity in the environment plays a prominent role for the outcome of predator–prey interactions (Savino and Stein 1982; 1989; Werner et al. 1983; Anderson 1984; Werner and Hall 1988; Turner and Mittelbach 1990; Eklöv and Diehl 1994; Persson and Eklöv 1995). Several of these studies demonstrate that structural complexity not only may affect species mortality rates by providing shelter in different forms but also may affect individual growth rate by offering a varying density and diversity of food resources (Crowder and Cooper 1982; Gilinsky 1984; Diehl 1993; Diehl and Eklöv 1995). This study documents that the quality and quantity of habitat structural complexity are of major importance for the distribution patterns of perch and pike. Below, I first discuss some potential mechanisms for the distribution patterns of perch and pike in relation to habitat structure. Then, I develop the implication of the behavioural mechanisms revealed in previous experimental studies for the predator–prey interactions between perch and pike (Eklöv 1992; Eklöv and Diehl 1994; Diehl and Eklöv 1995; Eklöv and Persson 1995). I show that the variation in the distribution patterns of perch and pike that occurred in the lake can mainly be explained by the strong behavioural interactions between predators and prey.

Structural complexity and habitat use of perch and pike

In Lake Degersjön, the highest macroinvertebrate biomass, and also the highest diversity of macroinvertebrates were found in the highest vegetation densities. However, the perch in my study strongly selected the intermediate vegetation density. These observations can be related both to foraging and antipredator strategies of perch. First, the foraging efficiency of perch has been shown to be inversely related to habitat structural complexity (Diehl 1988). At the same time, experimental studies suggest that the higher abundances of resources in vegetated habitats may possibly compensate for the decreased efficiency of foraging perch in that habitat, allowing benthivorous perch to grow at the same rate as perch foraging in open habitats (Diehl 1993). A similar result has been

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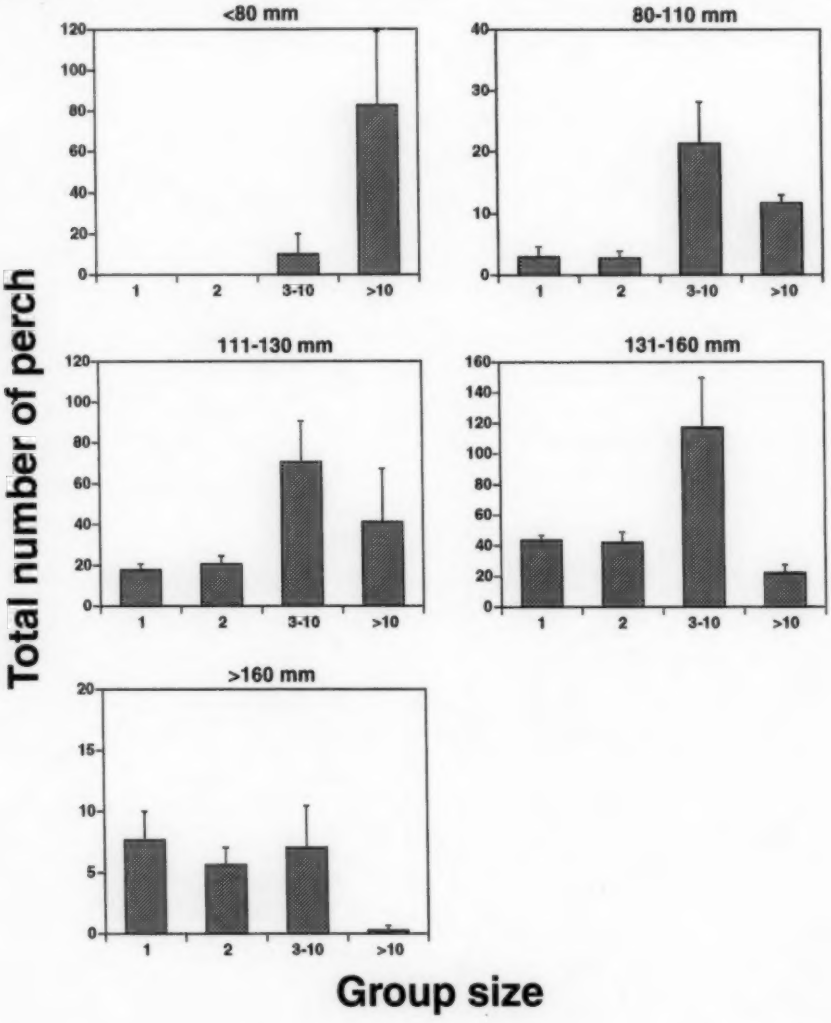
Fig. 9. Total perch numbers of each perch size class that occurred in different group sizes at locations A, B, and C 1990. Values are means ± SEs (3 replicates/group size level).



observed for bluegill sunfish (*Lepomis macrochirus*); bluegills foraging at an intermediate vegetation density level had a wider diet breadth and higher growth rate than bluegills foraging in habitats with no vegetation or in habitats with high vegetation densities (Crowder and Cooper 1982). Second, predation risk decreases with an increase in structural complexity (Anderson 1984; Persson and Eklöv 1995). This suggests that vulnerable prey fish in the presence of piscivorous predators should choose densely vegetated habitats to avoid predation, which also have been shown for perch in the size range 80–110 mm (Eklöv and Diehl 1994). In July, perch 80–110 mm were only found in the vegetated habitats, whereas larger size-classes of perch stayed more in open habitats. Thus, the preference for the intermediate vegetation density of perch could possibly be a tradeoff between maximizing foraging advantages and minimizing predation risk of perch.

Although all sizes of perch preferred the intermediate vegetation density, the different sizes of perch changed their overall habitat choice in different ways over time. The number of perch 80–110 mm collected by electrofishing declined dramatically from May to June, suggesting two possibilities: (1) movement into the pelagic zone or (2) mortality. The observed pattern is most likely explained both by perch 80–110 mm shifting into the densely vegetated habitat (Fig. 5), as a result of predator avoidance, but at the same time suffering from substantial predation (Fig. 3; see also Eklöv and Diehl 1994). A result supporting these hypotheses was that large perch and pike selected more open habitats (e.g., tree structure) located close to the vegetated habitats containing high densities of perch 80–110 mm during the summer months. A possible explanation as to why perch and pike use more open habitats close to vegetation is that this may minimize the negative effect of structural complexity on their foraging efficiency and at the same time maximize encounter

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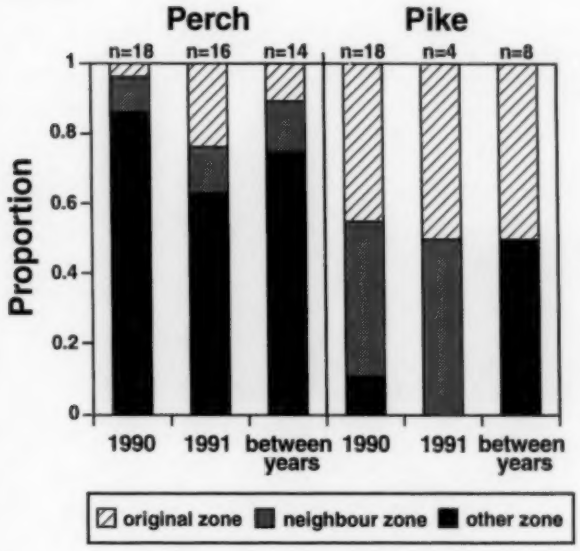
Fig. 10. Proportion of large perch and pike recaptured in the original zone (maximum range 100–440 m, neighboring zone (maximum range 101–565 m), or other zones (maximum range 286–2304 m) in 1990 and 1991 and individuals recaptured 1991 that were tagged 1990 (between years). *n*, Numbers of individuals recaptured.



frequency with prey staying in the refuge habitats. In support of this hypotheses, it has been shown that large perch and pike stay close to or move inside habitats that are used as refuges for prey fish (Eklöv and Diehl 1994; Eklöv and Persson 1995).

Large pike also preferred the tree structure habitat during the summer months. In fact, the pike captured by electrofishing and observed by snorkeling in or close to the tree structure habitat were the largest pike observed in the lake. Furthermore, pike size was inversely related to vegetation density, suggesting that the smaller pike stayed in dense vegetation to avoid predation. Preference for different habitats by pike has also been found to be size specific in another study, and especially pike <140 mm were often associated with dense submerged vegetation (Grimm 1994). Grimm (1994) suggested that the selection of densely vegetated habitats by small pike enabled them to avoid cannibalism from larger pike. The interaction between pike has also been found to depend on both the availability of prey and size of habitats (Eklöv 1992; Eklöv and Diehl 1994). In the latter study, large pike had effects on both growth rate and habitat use of small pike, and the larger pike were always found closer to the prey than the smaller pike, whereas the smaller pike moved into vegetation patches probably to avoid predation from larger pike. All these results point to direct interactions between pike individuals probably being cannibalistic, which makes the survival of smaller pike dependent on the size and density of the vegetated habitat.

Shoaling behaviour of perch

The group size of perch decreased with increasing vegetation density, suggesting that (*i*) group foraging was less profitable (or possible) in the vegetation or (*ii*) that increasing structural complexity decreased predation risk, decreasing the profitability of using the group as a defense against predators (see Pitcher 1986). Group behaviour has been found to be related to both predator detection and avoidance and to improve individual foraging rate (Pulliam 1973; Powell 1974; Clark and Mangel 1984, 1986; Eklöv 1992). By increased vigilance, individuals within a group can detect predators at longer distances and may also improve the use of information in the presence of scarce patchily distributed prey (Clark and Mangel 1986). My results showed that perch <80 mm preferred large group sizes (>10 individuals) whereas large perch preferred small group sizes (<10 individuals), and the two size-classes of perch never occurred in the same groups. It can be hypothesized that perch <80 mm occurred mainly in large groups, because small individuals are more susceptible to predation, and that the vulnerability of individuals to predation decreases with increasing group size (Pitcher 1986; Magurran 1990). The tendency for large perch to prefer smaller groups or to stay alone can be related to the fact that they switch to a sit-and-wait strategy in structurally complex environments (Eklöv and Diehl 1994; Eklöv and Persson 1995). Whether the effects of shoaling are positive or negative and if there is an optimal group size is ambiguous because an increase in group size may both increase or decrease individual growth rate depending on the social interactions within the group (Metcalf 1986; Cui and Wootton 1989; Eklöv 1992).

Piscivore movement patterns and the significance of different foraging strategies

Large perch showed small tendencies to be recaptured in the same zone on successive samplings, whereas pike were more disposed to stay in the same zone or in a neighboring zone. This suggests that large perch adopted a more active mode, and pike used a more sit-and-wait mode. In addition, the fact that pike stayed in the same zone or moved to the neighboring zone suggests that pike used a specific home range, which also have been found in other studies of pike (Halme and Korhonen 1960; Malinin 1969). The relative predation efficiency of large perch and pike have been found to depend on their different foraging strategies (Eklöv 1992; Eklöv and Diehl 1994; Eklöv and Persson 1995). In the absence of vegetation, large perch were favored by a group foraging strategy, whereas the efficiency of pike decreased with an increase in predator density as a result of interactions between individuals (Eklöv 1992). When prey refuges in the form of submerged vegetation are present, large perch switch from an active group foraging mode to an inactive solitary mode or stay in pairs in which they have a lower foraging efficiency than sit-and-wait foraging pike (Eklöv and Diehl 1994; Eklöv and Persson 1995). Large perch in Lake Degersjön stayed solitary or in small groups with other size-classes of perch, which indicates that they did not use a group foraging strategy in the littoral zone.

The observed activity pattern of large perch can be suggested to depend on the time scale used in the study. On a short time scale, large perch may appear to be inactive sit-and-wait predators (see Eklöv and Diehl 1994; Eklöv and Persson 1995), whereas on a long time scale, they appear to be active and move around the lake. Two explanations can be advanced to explain the functional significance of this time scale dependent activity pattern in perch. First, on a long time scale, large perch can use an active mode to more efficiently track spatial

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changes in resource levels. Second, on a short time scale, large perch may switch to a sit-and-wait mode if prey are located in or close to structured environments, such as littoral zone vegetation (Eklöv and Diehl 1994). In fact, large perch in Lake Degersjön were found to select high-density patches of perch 80–110 mm, a size range that is possibly subject to piscivorous predation (Bagenal 1977; Van Densen 1994; Persson et al. 1996). Furthermore, perch are active and social predators and are also morphologically adapted to an active mode (see Webb 1984; Eklöv and Persson 1995). Thus, the activity of littoral large perch in Lake Degersjön may also simply be a result of their life-style (sensu Sih 1987). An interesting circumstance in this context is the low exchange rate of large perch between the littoral and pelagic zones. Based on the lower foraging efficiency of large perch in structured versus nonstructured environments (Eklöv 1992; Eklöv and Diehl 1994), one would expect visits to the littoral zone to be sporadic by large perch. However, no tagged large perch were caught in the gill nets despite the fact that recapture of eight tagged individuals was expected based on the numbers of tagged and recaptured large perch in the littoral zone. Even if a total mixture of pelagic and littoral perch was not expected, the data shows that the interchange of large perch between the littoral and pelagic zones was low.

The implications of movement and habitat use of pike in terms of potential effects on the prey population are more easy to interpret because pike occur mainly in the littoral zone even if small numbers of large pike have been observed offshore (Diana et al. 1977; Diana 1980; Vøllestad et al. 1986). In the littoral zone, pike have been found to affect the abundance of prey ranging in sizes between 80 and 130 mm (Bagenal 1977; VanDensen 1994; Persson et al. 1996). The fact that large pike were located close to high density patches of perch 80–110 mm indicates that this size-class of pike select home ranges that coincide with high densities of prey. The home ranges of the largest pike lasted over years. The strong tendency of pike to stay close to high-density prey patches was also found in an enclosure experiment, where the largest pike were always found closest to the prey (Eklöv and Diehl 1994).

The habitat preferences of large perch and pike indicate that they used the same sites in the lake. Large perch and pike affect different size-classes of prey differently (Diehl and Eklöv 1995; Persson et al. 1996). For example, pike have been shown to have strong effects on the refuge use, survival, and growth of both 0+ and 1+ perch, whereas piscivorous perch only affected 0+ perch (Eklöv and Diehl 1994; Diehl and Eklöv 1995; Persson et al. 1996). Furthermore, in the absence of pike and in the presence of large-sized perch, 1+ perch moved out of the refuge, suggesting a potentially lower competition between size-classes of perch when pike is not present in the system (Diehl and Eklöv 1995).

In conclusion, my study suggests that the structural complexity in the littoral zone can both qualitatively and quantitatively change the interaction between piscivorous predators and their prey. Vegetation density appeared to be especially important in potentially mediating predator–prey interactions both by supporting macroinvertebrate food resource for benthivorous perch and as protection from piscivorous predation. Although I was not able to evaluate the predation impact of large perch and pike on prey fish, the spatial distributions of the different size-classes of perch and pike suggest a strong behavioural interaction between predators and prey. These results were supported by previous enclosure or pond experiments in which mechanistic explanations for the distribution patterns of perch and pike were given (Eklöv 1992; Eklöv and Diehl 1994; Eklöv and Persson 1995). My study in Lake Degersjön further suggests that the foraging efficiency of piscivorous perch and pike might be altered by interspecific interaction as a result of their tendency to exploit the same food resource patches. To be able to answer the question on how such interspecific effects are created and what potential effects they may in turn have on other species, manipulative studies using treatments with both predators present are necessary.

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Use of spectral analysis to estimate short-term periodicities in growth rates of brook trout *Salvelinus fontinalis*

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Abstract: We used spectral analysis to investigate the periodic components of relative growth rates (on a mass and length basis) in two groups of six young-of-the-year brook trout (*Salvelinus fontinalis*). Fish were measured every 2 days for 25 days according to a repeated measures design in time. The predominant frequency components were estimated at the end of a stepwise procedure involving the analysis of the multifrequential periodogram. We found mainly two superimposed periodic components that resulted in short rhythms; these had a period of about 5 and 10 days for the growth rate in mass and of about 4 and 6 days for the growth rate in length ($R^2 = 91\%$ for mass and 90% for length, both including an autocorrelation term). Repeatability of rhythms was assessed by ANOVA of the finite Fourier transform, which showed that the two groups of fish reared under the same experimental conditions exhibited growth rhythms that were not significantly different from each other. On that basis, we present a harmonic model which predicts short-term variation in growth rate of brook trout in mass ($R^2_{pred} = 0.73$, with an autocorrelation term) and in length ($R^2_{pred} = 0.72$).

Résumé : Nous avons utilisé l'analyse spectrale pour étudier les composantes périodiques des taux de croissance relatifs (en masse et en longueur) dans deux groupes de six ombles de fontaine (*Salvelinus fontinalis*). Les poissons furent mesurés tous les 2 jours durant 25 jours selon un plan expérimental en mesures répétées dans le temps. Les composantes en fréquence prédominantes ont été estimées au terme d'une procédure pas à pas croissante impliquant l'analyse du périodogramme multi-fréquentiel. Nous avons trouvé principalement deux composantes périodiques qui, en se superposant, produisaient des rythmes courts: leurs périodes étaient d'environ 5 et 10 jours pour le taux de croissance en masse et d'environ 4 et 6 jours pour le taux de croissance en longueur ($R^2 = 91\%$ pour la masse et 90% pour la longueur, en incluant un terme d'autocorrélation). La répétabilité des rythmes a été éprouvée par analyse de variance de la transformée de Fourier finie, qui a montré que les rythmes de croissance des deux groupes de poissons élevés dans les mêmes conditions expérimentales n'étaient pas significativement différents les uns des autres. Sur cette base, nous présentons un modèle harmonique pour prédire la variation à court-terme des taux de croissance de la truite mouchetée en masse ($R^2_{pred} = 0.73$, avec un terme d'autocorélation) et en longueur ($R^2_{pred} = 0.72$).

Introduction

Fish growth has long been recognized to be a periodic phenomenon (Planes 1993). Temporal scales over which rhythms in growth have been studied range from 1 day to 1 year (Leatherland et al. 1992). Most of the information available, however, pertains to the two extremes of this range. Seasonal periodicities with faster growth in the summer and slower growth in the winter is well documented for fish in temperate regions (Brown 1946; Fonds 1979; Wang and Houde 1994; Griffiths and Kirkwood 1995; Jensen and Berg 1995) and in tropical regions where fish growth follows the annual cycle of rainfall and drought (Clifton 1995; Louis et al. 1995). At a finer scale, circadian rhythms have been demonstrated for the calcareous deposits in fish otoliths (Pannella 1971; Taubert and Coble 1977; Campana and Neilson 1982). The width of rings formed in the otoliths has been related to daily increases of fish length and mass (Taubert and Coble 1977). Despite a wealth of information on fish growth (Donaldson et al. 1979), relatively few studies have suggested the existence of periodicities of a few days to a few weeks (Brown 1946; Pannella 1971, 1974; Campana 1984; Wagner and McKeown 1985; Farbridge and Leatherland 1987*a*, 1987*b*; Planes 1993). Scarcity of the information on short to intermediate periodicities (SIP) may be related to the tendency of investigators to express growth as a cumulative process over time and hence to mask its periodic characteristics (Wagner and McKeown 1985). Furthermore, secondary structures of long time series of growth data are often ignored and considered as part of the random error (Legendre and Dutilleul 1992). Finally, when several rhythms are combined, identification of distinct rhythmic components may require statistical tools that are not commonly used in fish biology (Girling 1995; Dutilleul 1995).

SIP may have fundamental and practical implications. For instance, experiments on fish growth, physiology, or behavior are often repeated at intervals ranging from a few days to a few weeks (Balm and Pottinger 1993; Holmes et al. 1994; Krohn and Boisclair 1994). Erroneous conclusions may be drawn when the physiological or behavioral responses studied vary, not because of treatment effects but resulting from rhythmic

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fluctuations. A better understanding of SIP may diminish the unexplained fraction of variance in physiological data (e.g., growth, feeding, respiration) and refine the comparison among experimental units. Feeding schedules used in pisciculture assume that under constant and quasioptimal water temperature, fish physiological and (or) behavioral dispositions to grow are relatively constant within intervals of a few weeks. The existence of SIP may suggest that appetite and anabolism also follow SIP, so these rhythms could be profitably used to optimize fish feeding strategies.

Assessment of the influence of SIP on our interpretation of biological processes first requires confirmation that the signals observed at an appropriate temporal scale are not just random errors. This confirmation occurs when a specific response is repeatable under the same environmental conditions and when the signal under observation is predictable. Consequently, the objectives of our study are (*i*) to evaluate the existence of short periodicities in the growth of brook trout (*Salvelinus fontinalis*), (*ii*) to assess the repeatability of the growth rhythms, and (*iii*) to develop descriptive and predictive models for the growth rates of that species.

Materials and methods

Experimental design

Young-of-the-year brook trout were obtained from a fish farm (Pêcheries Oka, Laurentian region of Quebec) to maximize homogeneity of fish size, age, and genetic characteristics. Twelve fish (20.56 ± 0.75 g wet mass (mean ± SD); 12.38 ± 0.59 cm total length (TL)) were randomly allocated to two 700-L aquaria (six fish per Living Stream LS-700) under constant and identical experimental conditions of water temperature (12 ± 0.5°C) and photoperiod (12 h light : 12 h dark). Fish were individually marked using 0.02 mm diameter platinum wires inserted at different positions of the fins or tail. They were fed commercial fish feed (Purina No. 5158) once daily at a ration level of 2.5% body mass·day^{-1} and were acclimatized 2 weeks before the beginning of the experiment on August 29, 1994. The experiment was planned as a repeated measures design in time (Crowder and Hand 1990). The length of the time series was fixed to 25 days (extent) and the sampling interval to 2 days (grain); the temporal scale defined by the 25-day extent and 2-day grain allows the detection of SIP ranging from 4 to 12.5 days. Every 2 days, each fish was thus lightly anaesthetized by immersion in a 1 mg·L^{-1} solution of MS-222 (tricane methanesulfonate) for a few seconds, then it was weighed (±0.01 g wet) and measured (±0.05 cm TL). This procedure was adopted to allow accurate measurements of length and mass, while minimizing the influence of handling stress on the fish.

Calculations

Fish growth rates were estimated as relative values on a mass and a length basis. Relative growth rate in mass was expressed as the mean rate of change in live mass (%·day^{-1}) following equation from Ricker (1979):

$$(1) \qquad \text{Relative growth rate in mass} = \frac{W - w}{T - t} \times \frac{100}{w}$$

where w and W represent the initial and final mass (g) for a time interval of length $T - t$. Relative growth rates in length were calculated in a similar way. The use of relative growth rates makes subtle changes in growth more readily discernible and facilitate comparison among groups (Weatherly and Gill 1987).

Relative growth rates were standardized to prevent the rhythm of a single fish from dominating the rhythm of the others. Standardization was carried out by centering each individual series of relative growth rates around its mean and dividing the centered data by the standard deviation of the series. The standardized relative growth rate (SRGR) in mass or in length was used in all calculations, except when explicitly mentioned otherwise.

Statistical analyses

A time-domain approach based on the repeated-measures ANOVA was adopted to test whether time, as a factor, affected the growth rates and whether the growth rates significantly differed between groups. This approach allows an overall comparison between groups in time for a given extent and grain. Frequency-domain techniques (spectral analysis) based on the analysis of the multifrequential periodogram and the ANOVA of the finite Fourier transform were used thereafter. The multifrequential periodogram was used to detect and estimate SIP in the growth rates. The ANOVA of the finite Fourier transform was used to test whether the two groups differed in the amplitude and phase of their growth rhythms. This approach allows a multiscale analysis by the consideration of frequency components of varying periods (Dutilleul 1997). Spectral analysis was completed by modeling the temporal trajectory of the growth rates as a harmonic process including an autocorrelation term when required.

Repeated-measures ANOVA

Normality of the data is required by the F test of the repeated measures ANOVA. Because of the autocorrelation issue in goodness-of-fit tests (Dutilleul and Legendre 1992), normality of the relative growth rates (in mass and in length) was assessed separately at each sampling time and not over all the time series, with individual fish as replicates. We used the Shapiro and Wilk (1965) test for its power in the absence of autocorrelation, as recommended in the literature.

We followed the modified univariate approach to the analysis of repeated measures by using Box's epsilon in correcting the ANOVA F tests (Crowder and Hand 1990). Time, the repetition factor, was of particular interest, as was the group-by-time interaction. Absence of interaction between time and group indicated parallel trajectory of the two groups over time, while the presence of interaction indicated nonparallel trajectories that may be due to a difference in amplitude, in phase, or both, for one or more rhythms. The model used (Winer 1971, p. 303) was

$$(2) \qquad Y_{ijt} = m + a_i + b_t + \mathrm{Ind}_{j(i)} + (ab)_{it} + e_{ijt}$$

where Y_{ijt} is the SRGR (in mass or in length) on day t for fish j in group i, m is the overall mean of the growth rate, a_i is the main effect of group i, b_t is the main effect of time t, $\mathrm{Ind}_{j(i)}$ is the effect of fish j nested within group i, $(ab)_{it}$ is the group-by-time interaction, and e_{ijt} is the random error.

The group and time factors were crossed and considered fixed; replicates were provided by the individual fish nested within the group.

Periodogram analysis

We followed Priestley's (1981, pp. 616–617) recommendations for the analysis of mixed spectra (i.e., when the time series is made up as a finite sum of sine and cosine waves plus an autocorrelated random error). Hidden periodicities were detected and estimated by periodogram analysis first, then they were fitted in a trigonometric model. Residuals were checked for remaining autocorrelation. The idea underlying the periodogram analysis is that the larger the amplitude of a cosine wave fitted to a time series by least squares, the larger the periodogram value at the corresponding period or frequency. This resulted in Schuster's (1898) periodogram statistic whose evaluation is generally limited to the Fourier frequencies that correspond either to the temporal mean (no cycle) or to an integer number of cycles over the observed time series, ranging from one to the integer part of half the length of the series.

Recently, Dutilleul (1990) proposed the multifrequential periodogram statistic for the detection of periodic signals in time series. For

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a given number of frequencies, Dutilleul's statistic is defined as the sum of squares of the trigonometric model made of the corresponding cosine and sine waves and fitted by least squares to the observed series. Among other things, the limitation to the Fourier frequencies in evaluating the statistic is relaxed, and this periodogram analysis allows the detection of periodicities that are close to one another in a time series. As the actual number of periodicities is generally unknown a priori, a stepwise procedure has been proposed to estimate it (Dutilleul 1990). One more frequency is considered at each step of the procedure, which is based on the resulting increase in the sum of squares of the best trigonometric model fitted by least squares (i.e., in the maximized value of the multifrequential periodogram statistic). Given the number of frequencies retained at the end of the procedure, the frequencies that maximize the periodogram statistic provide the estimated periodicities. The methodology derived in Dutilleul (1990) has been introduced by Dutilleul and Till (1992) and Legendre and Dutilleul (1992); it is now presented in Dutilleul (1997) and Legendre and Legendre (1997).

The analysis of the multifrequential periodogram and the related stepwise procedure were carried out for the individual series of *S. fontinalis* SRGR and for the mean series computed per group and that pooled over groups. This pooling was justified based on the results of the ANOVA of the finite Fourier transform.

Analysis of variance of the finite Fourier transform

When replicated time series are collected in an experimental design according to some treatment factor or some classification criterion (e.g., the group here), the analysis of variance of the finite Fourier transform of the replicated series allows the assessment of differences in the frequency domain among the levels of the factor or criterion considered (Brillinger 1973; Dutilleul 1990). Replicated time series are provided here by individual series of *S. fontinalis* SRGR (in mass or in length) from fish of the same group. The analysis of variance of the finite Fourier transform thus completes the periodogram analysis by a sequence of F tests performed at the Fourier frequencies, denoted ω_k hereafter. The corresponding sequence of hypotheses is H_{0k}: at frequency ω_k, the SRGR, as a time-series process, does not differ between groups in amplitude and in phase, against the alternative. This sequence of tests and hypotheses results from the orthogonal decomposition of the group-by-time interaction in eq. 2 by application of the finite Fourier transform (Dutilleul and Till 1992; see Deswysen et al. (1989) and Schelstraete et al. (1992) for further detail). After finite Fourier transform, the group main effects are the only effects remaining in the ANOVA model, which is univariate at the zero frequency (i.e., the temporal mean over the 25 days) and bivariate at all the other frequencies. The finite Fourier transform benefits from suitable statistical properties (i.e., normality and independence) (Brillinger 1975).

Modeling

The temporal trajectory of the SRGR was described by a trigonometric model built up as a sum of sine and cosine waves (deterministic part) at the frequencies estimated by analysis of the multifrequential periodogram, on which autocorrelated or purely random errors (stochastic part) are superimposed. If $\{Y_t ; t = 1, 2,..., n\}$represents a time series of n SRGRs the model used is

$$(3) \qquad Y_t = \sum_{l=1}^{L} \{a_l \cos(\hat{\omega}_l t) + b_l \sin(\hat{\omega}_l t)\} + e_t$$

where $\{\hat{\omega}_l ; l = 1, 2,..., L\}$denote the frequency estimates retained at the end of the stepwise procedure in the periodogram analysis and $\{e_t; t = 1, 2,..., n\}$are the errors, purely random or not, depending on the autocorrelation found in the residuals after fitting the frequency components. Equation 3 was also used for short-term prediction.

Low-frequency estimates (i.e., of less than two cycles over the 25 days) were not included in the deterministic part of the model because they corresponded to peaks in the autocorrelation part of the spectrum, so they did not correspond to rhythms. The other frequency components were fitted in a model where the autocorrelation of the random errors was assessed. It is important to take temporal autocorrelation into account, when significant, because (1) otherwise, it affects the estimation of the slopes a_l and b_l by increasing their variance and (2) autocorrelation is the basis of forecasting (e.g., Box et al. 1994), by increasing the capacity of the model to predict at short term.

The Shapiro–Wilk test of normality was carried out by SAS procedure UNIVARIATE, option NORMAL (SAS Institute Inc. 1990). The analysis of the multifrequential periodogram and the related stepwise procedure were performed using the PerioMod software version 1.2. Copies of PerioMod (Macintosh and PowerPC versions) are available, with technical notes, from the second author upon request. Analysis of variance of the finite Fourier transform was carried out by PROC SPECTRA of SAS/ETS® version 6 (SAS Institute Inc. 1988), combined with PROC GLM of SAS/STAT® version 6 (SAS Institute Inc. 1989). For the modeling part, we used PROC AUTOREG of SAS/ETS® version 6 (SAS Institute Inc. 1988).

Results

Individual relative growth rates ranged from –4.50 to 8.43% body mass·day^{-1} and from –0.76 to 2.63% body length·day^{-1}. Visual inspection of individual fish profiles suggested the presence of rhythms in both groups during the course of our study (Fig. 1). These profiles further suggested that, despite differences among individual fish trajectories, group trajectories seemed to follow the same general rhythmic pattern in relative growth rate in mass (Figs. 2A and 2C) and, to a lesser degree, in the relative growth rate in length (Figs. 2B and 2D). These observations were supported by our periodogram analysis and the ANOVA of the finite Fourier transform.

Repeated-measures ANOVA

Normality was not rejected significantly more often than expected for the 0.05 level. Group main effects were not significant (Table 1), indicating that relative growth rate in mass and in length did not differ between the two groups of fish across the 25 days. Time had a significant effect on SRGR in mass but not in length (Table 1). This probably resulted from inflation of the error term in eq. 2 that incorporates the true experimental error and the interaction between time and fish nested within the group. This Error (time) sum of squares obtained for SRGR in length (124.3) was larger than for SRGR in mass (68.02) (Table 1). Furthermore, the interaction between time and fish seemed to be more prominent for relative growth rate in length than for mass. Peaks of growth in length appeared on different days depending on the fish (Figs. 1C and 1D), whereas the growth of individual fishes in mass tended to follow more similar patterns over time (Figs. 1A and 1B). The group-by-time interaction was not significant for both SRGRs (Table 1). This indicated that the temporal trajectories of the group mean SRGRs were parallel (Figs. 2C and 2D).

Periodogram analysis

The frequency components most commonly shared by the individual fish were about four (22%), five (17%), and two (14%) cycles over 25 days in the SRGR in mass and two (31%), five (22%), and six (17%) cycles over 25 days in the SRGR in length (Table 2). Periodogram analysis of the mean SRGR in mass, once pooled over groups, detected frequency components of 4.74, 2.52, and 1.11 cycles over

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Fig. 1. Individual curves of relative growth rate in mass for brook trout (*Salvelinus fontinalis*) in groups 1 (A) and 2 (B) and of relative growth rate in length in groups 1 (C) and 2 (D).

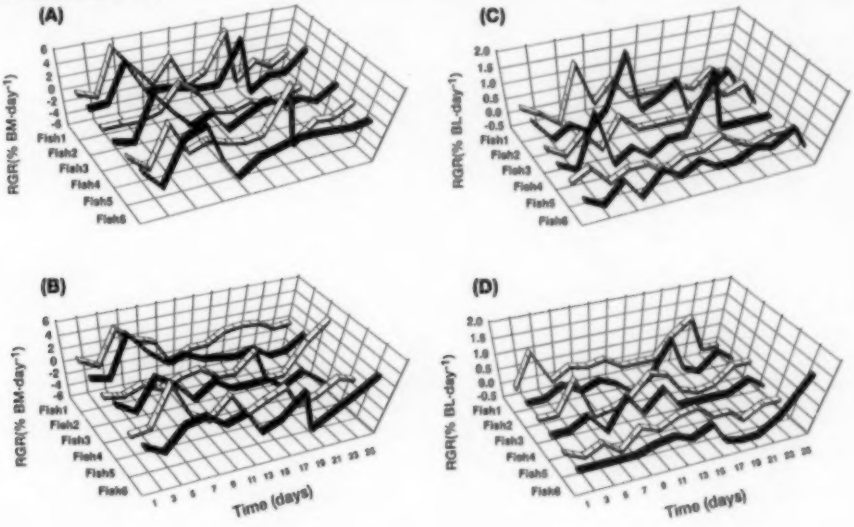
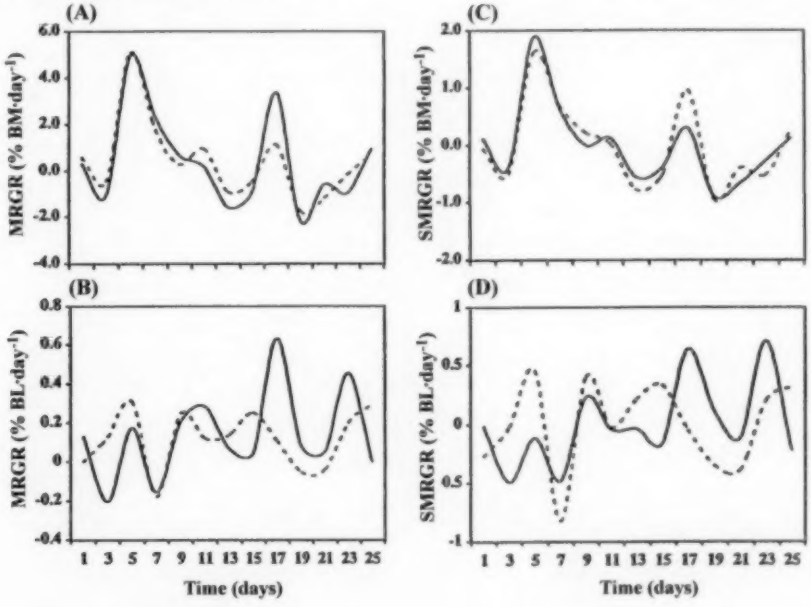


Fig. 2. Group mean curves for the relative growth rates in mass (A) and in length (B) and group mean curves for the SRGR in mass (C) and in length (D). Solid lines, group 1; broken lines, group 2.



25 days, which jointly explained 91% of the total variation (Table 3). Periods associated with the first two frequencies are about 5 and 10 days; frequency component 1.11 comes from a peak in the continuous part of the spectrum, so it corresponds to autocorrelation rather than a rhythm. For the SRGR in length, predominant frequency components of 5.98, 3.93, and 0.50 cycle(s) over 25 days were detected in the pooled mean series; they jointly represent 90% of the total variation (Table 3). Frequency components 5.98 and 3.93 correspond to superimposed rhythms with periods of 25/5.98 ≈ 4 days and 25/3.93 ≈ 6 days, while frequency component 0.50 did not correspond to a rhythm for the same reason as above. The presence of three statistically significant frequencies was illustrated by three peaks in the contour plot of the bifrequential periodogram statistic for both SRGRs (Fig. 3).

Analysis of variance of the finite Fourier transform

The two groups of fish did not show any significant difference in either SRGR for any Fourier frequency (Table 4). Rhythms can be assumed to be repeatable because there was no evidence

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Table 1. Repeated measures ANOVA for the SRGR in mass and length.

Source	df	MS	F	$P > F$	G–G[a]	H–F[a]
			Between fish			
Mass						
Group	1	<0.01	<0.01	0.9954[b]		
Error	10	2.20				
Length						
Group	1	0.01	0.08	0.7822[b]		
Error	10	0.15				
			Within fish			
Mass						
Time	12	6.13	10.81	0.0001	0.0001	0.0001
Time × group	12	0.20	0.36	0.9747	0.8308	0.9280
Error (time)	120	0.57				
Length						
Time	12	1.04	1.01	0.4469	0.4255	0.4469
Time × group	12	0.60	0.58	0.8582	0.7280	0.8582
Error (time)	120	1.04				

[a]Greenhouse–Geisser Epsilon for SRGR = 0.3244 (mass), 0.4421 (length); Huynh–Feldt Epsilon for SRGR = 0.6101 (mass), 1.0946 (length). The values for G–G and H–F are the corresponding adjusted probabilities of significance.

[b]The absence of significant differences between the overall group means computed over the 25 days is a result of the standardization (see text). Using the raw data, the two groups do not differ significantly either in mass ($P > F = 0.1282$) or in length ($P > F = 0.0758$).

Table 2. Frequencies estimated at the end of the stepwise procedure involving the analysis of Dutilleul's (1990) multifrequential periodogram for the time series of individual SRGR in mass and length.

	Frequency 1		Frequency 2		Frequency 3		Periodogram value	
Fish No.	Mass	Length	Mass	Length	Mass	Length	Mass	Length
Group 1								
1	4.83	5.09	2.44	5.69	1.00	1.38	10.47	10.95
2	2.40	4.56	4.52	1.82	6.00	3.10	10.51	11.17
3	1.34	5.44	2.94	1.24	3.85	2.00	11.66	11.12
4	4.90	6.00	2.25	2.15	1.33	4.34	10.66	10.40
5	4.49	4.97	6.00	1.87	2.82	3.38	10.48	10.00
6	2.60	5.73	3.56	2.06	1.12	5.00	11.20	11.59
Group 2								
1	2.29	2.78	6.00	6.00	1.19	1.89	10.98	10.93
2	0.90	4.27	3.90	2.94	4.54	1.56	10.41	11.97
3	3.14	2.57	4.04	6.00	1.89	3.56	10.99	11.00
4	0.57	5.72	4.57	4.92	3.76	1.88	11.73	11.44
5	4.28	1.86	0.94	5.44	4.21	0.84	10.54	10.32
6	2.93	1.84	4.76	1.92	1.20	5.42	10.46	11.28

Note: The total sum of squares was 12.00

of difference between groups in the amplitudes and phases of their SRGR rhythms. Fish subjected to the same experimental conditions thus exhibited similar rhythms in growth rates both in mass and in length.

Modeling

The trigonometric model (eq. 3) was fitted to the pooled mean SRGR, with $\hat{\omega}_1 = 4.74$ and $\hat{\omega}_2 = 2.52$ for mass and $\hat{\omega}_1 = 5.98$ and $\hat{\omega}_2 = 3.93$ for length. The autocorrelation structure of residuals, as retained by AUTOREG, was first-order autoregressive for the SRGR in mass; the autocorrelation estimate at first lag (i.e., between one observation and the next 2 days later) was 0.46, which indicated that the random errors superimposed on the periodic components were positively autocorrelated. Residual autocorrelation was absent from the series of SRGR in length. The resulting models described well the SRGR, both in mass ($R^2 = 0.78$) and in length ($R^2 = 0.89$) (Fig. 4). This R^2 (called Reg RSQ by AUTOREG) is the classical measure of goodness-of-fit in regression (Draper and Smith 1981); it measures how the model fits the data by using only the structural or deterministic part (cosine and sine waves here). From a predictive perspective, when the focus is on how the current value can be predicted in the course of the experiment by using the structural part of the model and the past values, a measure of "goodness-of-prediction" (denoted R^2_{pred} here and called Total RSQ by AUTOREG) must be used; both models

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Table 3. Frequency components estimated at the end of the stepwise procedure involving the analysis of Dutilleul's (1990) multifrequential periodogram for the time series of group mean SRGR in mass and length.

	Group 1	Group 2	Groups 1 and 2	Period (days)
Step 1				
Mass	2.56	1.10†	2.52 (0.0049)‡	≈10
Length	5.72	5.30	3.93 (0.0224)‡	≈6
Step 2				
Mass	4.74	4.71	4.74 (0.0922)	≈5
Length	0.79†	2.44	5.98 (0.0423)	≈4
Step 3				
Mass	1.11	2.48	1.11 (0.1313)†	na
Length	4.54	5.29	0.50 (0.0566)†	
Periodogram value				
Mass	5.86	5.56	5.60	
Length	1.30	1.53	0.94	
Total sum of squares				
Mass	6.60	6.06	6.13	
Length	1.57	1.70	1.04	

†These frequency components correspond to peaks in the continuous part of the spectrum. They correspond to autocorrelation rather than rhythms.

‡For groups 1 and 2, the frequency estimates are followed by the corresponding probability-to-retain value of the stepwise procedure, which are given in parentheses. Groups 1 and 2 stand for the mean SRGR series pooled over the two groups.

continue to perform well from this respect with $R^2_{pred} = 0.73$ for SRGR in mass and $R^2_{pred} = 0.72$ for SRGR in length.

Discussion

Growth trajectories

Brook trout displayed short-term rhythms in growth rates in mass and in length. We observed a considerable variability among individual growth trajectories of fish, especially in the relative growth rate in length. Despite the interindividual differences in growth rate, we found a striking similarity between groups in the amplitude and phase of the mean growth trajectories. The interindividual variability may be due to social hierarchies within group, where dominant fish consumed more food and grew more (higher amplitudes) and faster (phase advance) than subordinates. In fact, we observed that dominant fish followed the same trajectory in both groups. Fish 4 and fish 1, which were dominant in groups 1 and 2, respectively, showed similar trajectories in the relative growth rate in length, reaching their first peak earlier than the other fish (Figs. 1C and 1D). The pattern of growth in mass of dominant fish was more or less similar, as they reached a peak on day 5 (i.e., before the other fish) and then fluctuated smoothly until day 25 (Figs. 1A and 1B).

Existence of rhythms

Two main rhythms of 5- and 10-day periods were identified in both groups for the relative growth rate in mass. Two main rhythms were also identified in the relative growth in length, with 4- and 6-day periods. If we had been to rely only on visual inspection of the graphs (Figs. 2A and 2B), we would have pointed out a 9- or 10-day periodicity in the growth rate in mass and a 6- or 7-day periodicity in the growth rate in length; the shorter periodicities of 4 and 5 days detected by the periodogram analysis would have run undetected. The multifrequential periodogram allows the explanation of a larger fraction of the variance and a more complete assessment of the rhythmic nature of growth phenomena. It is particularly helpful for the detection of rhythms composed of two or more periodic components that are close to one another and for the estimation of noninteger frequency components. For instance, the three dominant frequency components pointed out by Schuster's periodogram for our pooled mean SRGR series provided an R^2 of 0.68 for mass and 0.84 for length, compared with 0.91 and 0.90 with the multifrequential periodogram; the corresponding Fourier frequencies were, in ascending order of explanation, frequencies 1, 5, 4 and 6, 4, 1, respectively. We also analyzed published data of growth rate in coho salmon (*Oncorhychus kisutch*; Farbridge and Leatherland 1987*a*). Although periodicities in the growth rate in length were not distinct by visual inspection of time plots, the multifrequential periodogram showed two superimposed periodicities of 8 and 10 days ($R^2 = 0.70$).

Our study is not the first that suggests the presence of intermediate periodicities. Rhythms of 2 to 4 weeks in growth rates were already found by other researchers and were associated with lunar and semilunar cycles: Brown (1946) found a 2-week cycle in the growth rate in mass of 2-year-old brown trout (*Salmo trutta*); Wagner and McKeown (1985) found a 3- to 4-week cycle in the growth rate in mass of the rainbow trout (*Oncorhynchus mykiss*); Farbridge and Leatherland (1987*b*) showed that the rainbow trout and the coho salmon both displayed rhythmic patterns of growth in mass with 10- to 14-day periodicity. Planes (1993) suggested the existence of a 29-day periodicity in the growth in length of larval and juvenile sea bass (*Dicentrarchus labrax*). Otoliths also showed evidence of biweekly patterns of growth (Pannella 1971, 1974; Campana 1984). The 10-day periodicity that we found in the relative growth rate in mass may correspond to the 10- to 14-day rhythm reported by Farbridge and Leatherland (1987*a*, 1987*b*).

To our knowledge, the short periodicities of 4–6 days that we detected in fish growth rates have not been recognized previously. Although the time series were short (25 days), less

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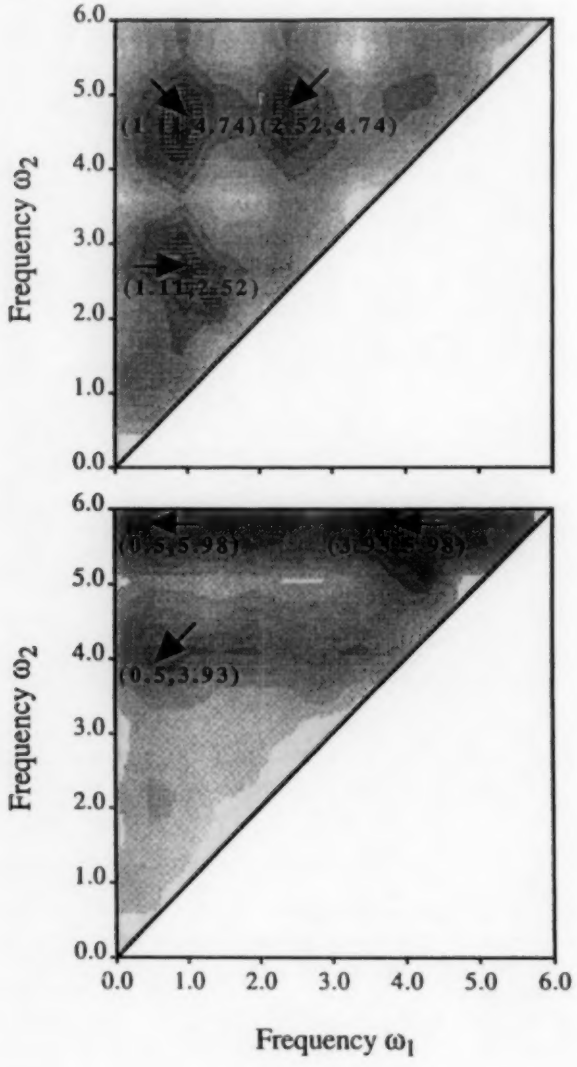
Fig. 3. Bifrequential periodogram plot for the mean curves of SRGR in mass (A) and in length (B) computed across the two groups. The statistic plotted is the sum of squares of the trigonometric model made of two frequency components and fitted to the series by least squares (Dutilleul 1990). The three peaks in each plot correspond to the three predominant frequency components taken two by two.



than five cycles is sufficient for the multifrequential periodogram to detect noninteger frequencies and separate frequencies close to one another for a not too low signal-to-noise ratio (Dutilleul 1996). In our study, the short cycles were repeated about four to six times. Only the intermediate periodicity corresponding to frequency 2.52 could be debatable since the cycle is repeated only twice. The stepwise procedure showed, however, that this frequency component was highly significant.

Our study was not aimed at demonstrating the causes or the nature of SIP in the growth rates of brook trout. We might, however, speculate on one or another causal factor. SIP could be exogenous rhythms entrained by factors related to data collection. This hypothesis may be excluded since the experiment was conducted indoors in a closed environment, and the experimental conditions (photoperiod, temperature, feeding time, feed ration) were kept as constant as possible. Although small variations of ±0.5°C in water temperature occurred, they were not found to be correlated with fish growth rate (R^2 = 0.04). In addition, the experiment was running exclusively in the laboratory so that entraining by human activity was also excluded. Therefore, SIP might be endogenous in nature. They might have evolved in response to environmental cycles such as water temperature or precipitation cycles. Periodicities of about 3–7 days were indeed reported for air temperature and precipitation in temperate regions (Landsberg et al. 1959). Other possible Zeitgebers could be biological factors such as food availability and social interactions. More information is needed on short-term fluctuations in the environmental and biological variables affecting fish in their natural habitats. Finally, unlike circadian, lunar, and seasonal rhythms, SIP might not follow any geophysical cycle. They could be a manifestation of intrinsic changes in fish physiology (i.e., non-circa-infradian rhythms). The refractory period between consumption and growth (i.e., the time taken by a fish to convert food consumed into proteins and pulses of growth) could serve as a physiological basis for these biorhythms. Spurs in growth could result from a switch in processing resources between different physiological processes. For instance, periodicities of 3.5–7 days have been reported in human mitotic activity (Blank et al. 1995; Focan et al. 1995). More studies of short-term variations in fish physiological variables, such as protein synthesis, ammonia excretion, Ca and amino acids uptake, and hormonal activity, are needed to explain the nature of SIP. Furthermore, variation in rhythms among species needs to be documented, as well as their variations with water temperature,

Table 4. Analysis of variance of the finite Fourier transform of the individual time series of SRGR in mass and length, at the Fourier frequencies ω_k = 1, 2, ..., 6 (no. of cycles over 25 days).

Fourier frequency ω_k	Statistic $F(\omega_k)^\dagger$	$P > F(\omega_k)$
1		
Mass	0.6229	0.5580
Length	1.0732	0.3819
2		
Mass	0.1245	0.8844
Length	0.4752	0.6365
3		
Mass	0.5263	0.6079
Length	0.3876	0.6895
4		
Mass	0.1008	0.9051
Length	0.0097	0.9904
5		
Mass	0.0523	0.9494
Length	1.8613	0.2106
6		
Mass	1.2873	0.3223
Length	0.0904	0.9144

$^\dagger F$ statistic equivalent to Wilks' lambda.

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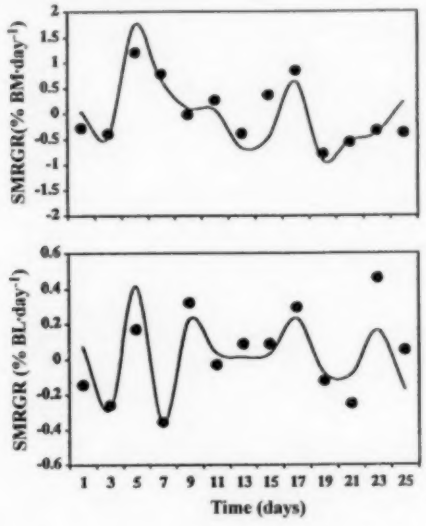
Fig. 4. (A) Observed SRGR in mass (solid circles) and growth rates in mass (curve) as described by the trigonometric model (R^2 = 0.89): $y_t = 0.0192 - 0.193\cos(5.98t) + 0.183\sin(5.98t) + 0.107\cos(3.93t) - 0.148\sin(3.93t) + e_t$. (B) Observed SRGR in length (solid circles) and growth rates in length (curve) as described by the trigonometric model ($R^2 = 0.78$): $y_t = 0.0140 + 0.279\cos(4.7t) + 0.545\sin(4.7t) - 0.232\cos(2.5t) - 0.541\sin(2.5t) + e_t$. See text for further details.



season, photoperiod, and the amount of food consumed by the fish.

Repeatability of rhythms

Under the same experimental conditions, the two groups of fish exhibited similar rhythms with periodicities falling in a range of 4–10 days. The repeated-measures ANOVA showed that, over the 25 days of the experiment and for a 2-day sampling interval, the mean growth trajectories of the two groups were not different. The multiscale comparison performed frequency by frequency (at a time span equal to the corresponding period) by the ANOVA of the finite Fourier transform did not provide any difference either. Repeatability of fish growth rhythms suggests that the SIP might be characteristic of the fish chronome. Variation in photoperiod, water temperature, feeding regime, etc., might alter the period, amplitude, and phase of the rhythms, so it is important to distinguish changes induced by the treatment from those caused by the physiological status of the fish.

Repeatability of rhythms was a key issue to be solved prior to future studies on SIP in fish growth rates. If the rhythms had been different under similar experimental conditions, treatment effects might have been confounded with the variability of the rhythm within the fish. Although a general pattern for SIP in fish growth rate might be predetermined or inherited, the characteristics of this pattern might change with the environmental conditions. The genetical and environmental contributions to growth rhythms and the selective advantage of such rhythms fall, however, beyond the scope of this paper.

Modeling of rhythms

We do not pretend to present a growth model for the brook trout. The trigonometric model (eq. 3) was found suitable to describe and predict short-term variation in fish growth rate under our experimental conditions. The model fitted the growth rate data (R^2 = 0.78 for mass and 0.89 for length) slightly better than it predicts any datum within the extent of the experiment from the previous data and the periodic components detected (R^2_{pred} = 0.72 for mass and 0.73 for length).

Our purpose was clearly to focus on short-term forecasting and not to use the model for predicting future SRGR on the long term (e.g., 10–20 days ahead), which may be erroneous because the developmental changes that the fish undergo with time may affect rhythm characteristics. Also, in practice, the frequency estimates must be updated over time, that is, frequencies $\hat{\omega}_1$ and $\hat{\omega}_2$ estimated over days 1–25 may have to be substituted on day 30, for example, by new frequencies $\hat{\omega}_1'$ and $\hat{\omega}_2'$ estimated over days 6–30.

Our trigonometric model is different from the previous models proposed (e.g., Hardeland 1995; Morgan and Minors 1995) in two ways. First, the predominant frequencies were estimated by the multifrequential periodogram, without a priori assumptions. Second, for given frequency estimates, the linear regression coefficients were estimated by taking into account the temporal autocorrelation in the random errors, when present. In the latter case, maximum likelihood was used instead of ordinary least squares.

Implications for the future

Our work supports the existence of statistically predictable patterns of growth and further suggests that fish physiological conditions can vary significantly among days. Implications of short-term rhythms in growth rate for the physiological and behavioral activities of the fish should be investigated further. If fish characteristics vary periodically every 4–6 days, experiments that are repeated at intervals ranging from few days to few weeks (e.g., on fish growth, physiology, or behavior) should be started at the same phase of the cycle. Our observations indicated that growth rate could be threefold higher at the peak than at the trough of the growth cycle. Relating biological and physical phenomena to specific periodicities will allow a better understanding of the nature of the rhythms observed, and modeling these rhythms may permit us to correlate fish growth or physiological status with environmental variables.

The present study has several potential implications on the applied side. Predictive models including SIP in growth and feeding could be useful, especially in pisciculture where young fish are maintained for 1 or 2 years. The model may then have to be adapted over time, for instance, every month. Food management could be more efficient if a greater amount of food was given in the rapid-growth part of the cycle and a smaller amount in the slow-growth part. Otherwise, fish may consume the same amount of food during the slow-growth phase as during the fast-growth phase but allocate energy for processes other than growth in mass or in length.

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Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*

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Abstract: Eleven tri- and tetra-meric motif microsatellite loci were identified in a lake sturgeon genomic library and were tested against the six North American species of *Acipenser* (lake sturgeon, *A. fulvescens*; shortnose sturgeon, *A. brevirostrum*; white sturgeon, *A. transmontanus*; green sturgeon, *A. medirostris*; Atlantic sturgeon, *A. oxyrhynchus oxyrhynchus*; gulf sturgeon, *A. o. desotoi*) and the two species of *Scaphirhynchus* (pallid sturgeon, *S. albus;* shovelnose sturgeon, *S. platorhynchus*) using four to six individuals of each species. Eight loci were amplified equally well in all eight species, and the remaining three loci were amplified in two, four, and seven species, respectively. Of the eight loci that worked in all species, one was monomorphic in all species, while the other seven were polymorphic in three to eight species. Single repeat differences in these tri- and tetra-meric repeat motifs can be readily scored on 4% Metaphor agarose gels stained with ethidium bromide. In addition, dosage for those loci exhibiting four gene doses can also be readily scored with this technique. These loci provide a much needed group of genetic markers, detectable with non-invasive sampling (blood, barbel, or fin), that should work well in threatened and endangered species of sturgeon worldwide.

Résumé : Onze loci de microsatellites présentant un motif tri- et tétramérique ont été observés dans une banque génomique d'esturgeon jaune et ont été testés par rapport à six espèces d'*Acipenser* d'Amérique du Nord (esturgeon jaune, *A. fulvescens*; esturgeon à museau court, *A. brevirostrum*; esturgeon blanc, *A. transmontanus*; esturgeon vert, *A. medirostris*; esturgeon noir, *A. oxyrhynchus oxyrhynchus;* esturgeon du golf, *A. o. desotoi*) et les deux espèces de *Scaphirhynchus* (esturgeon pâle, *S. albus*; esturgeon scaphirhynque, *S. platorhynchus*) en utilisant 4-6 individus de chaque espèce. Huit loci ont été bien amplifiés de manière égale, chez les huit espèces, et les trois loci restants ont été amplifiés chez deux, trois et sept espèces respectivement. Des huit loci présents chez toutes les espèces, un était monomorphique chez toutes les espèces, tandis que les sept autres étaient polymorphiques chez trois des huit espèces. Les différences de répétition unique dans ces motifs répétitifs tri-et tétramériques peuvent être facilement observées dans des gels d'agarose Métaphor à 4 % colorés au bromure d'éthidium. De plus, cette technique permet d'établir rapidement le dosage pour ces loci présentant quatre doses géniques. Ces loci fournissent un groupe de marqueurs génétiques dont on avait grand besoin, qui sont détectables par échantillonnage non invasif (sang, barbillon ou nageoire), qui devraient donner un excellent rendement chez les espèces esturgeons menacées et en danger d'extinction dans le monde.
[Traduit par la Rédaction]

Introduction

The order of fishes Acipenseriformes consists of two living families: Acipenseridae (the sturgeons) and Polyodontidae (the paddlefishes). Within Acipenseridae, the genera *Huso*, *Scaphirhynchus*, and *Pseudoscaphirhynchus* consist of two or three species each, while the genus *Acipenser* has more than 15 species. Worldwide nearly all sturgeon species are threatened or endangered (Birstein 1993).

Like other freshwater fish, sturgeon populations have suffered from overfishing, barriers to migration, loss of spawning habitat, and the deterioration of water quality. Recent efforts to improve water quality and aquatic habitats in North America have led to an interest in the restoration of these fishes. The International Conference on Sturgeon Biodiversity and Conservation held at the American Museum of Natural History in July 1994 helped to focus scientific attention on the precarious state of many sturgeon populations and the need for new methods to study these long-lived animals (Birstein et al. 1997).

Genetic research on sturgeons has been limited to a few studies on chromosomal numbers and cellular DNA content (Birstein et al. 1993), allozymes (Phelps and Allendorf 1983; Bartley et al. 1985), and mitochondrial DNA (Ferguson et al. 1993 and references cited therein). The sturgeon's extended age to maturity (5 to more than 20 years), extended period between spawning cycles (2 to more than 10 years), and their residence in deep waters of large lakes and rivers make them difficult species for genetic investigations of behavior, selective breeding, or population structure. Chromosomal and DNA content studies (Birstein and Vasilev 1987; Birstein et al.

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1993; Blacklidge and Bidwell 1993; Fontana 1994) divide the species into three groups of $2N$ = ~120, ~240, and ~360–500. These results, along with chromosomal data on related taxa, suggest that one polyploid event may have occurred in the evolution of the order Acipenseriformes with several more independent polyploid events in the evolution of the family Acipenseridae.

Codominant Mendelian markers are necessary for definitive studies to be conducted (*i*) to determine the genomic structure of sturgeon species with different chromosome numbers, (*ii*) to describe population structure of species, and (*iii*) to measure the effects of ongoing stocking programs on the genetics of native populations. Traditional allozyme studies are difficult to perform on sturgeon because of the need to sample eye, heart, liver, or kidney tissues, causing mortality of fish that are threatened or endangered.

Recent studies in a number of organisms have shown that the class of variable number tandem repeat (VNTR) loci with simple sequence repeat (SSR) motifs (two to five base pairs (bp); often termed microsatellite loci) offer the advantages of high levels of allelic variation per locus and simplified tissue sampling requirements for their analysis. Microsatellite loci often have more polymorphic loci and more alleles per polymorphic locus than allozyme loci. In addition, microsatellite loci can be scored from tissues non-destructively sampled (e.g., muscle, fin, hair, blood, feces, scale, feather) and preserved by freezing, drying, or stored in alcohol or lysis buffer. These loci have provided a wealth of data for many different types of genetic studies, such as parentage analysis in chimpanzees (Morin et al. 1994) and bees (Estoup et al. 1995), population studies in brown trout (Estoup et al. 1993) and black bears (Paetkau and Strobeck 1994), and genome mapping in mice (Dietrich et al. 1992) and humans (Luty et al. 1990).

Microsatellite loci in lake sturgeon were investigated to find new simple Mendelian markers useful for future population and breeding studies. In this report, 11 microsatellite loci and their respective amplification primers are described that were cloned from a lake sturgeon genomic library. These primers were then tested on extracted DNA from all eight North American species of sturgeon from the genera *Acipenser* and *Scaphirhynchus* to determine the homology of these primers in related taxa.

Methods

Collections

Portions of fin (0.5–1.5 cm^2) were excised, placed in 100% ethanol, and either refrigerated or stored at room temperature for 1–6 months. Fin samples were taken from 4 to 11 individuals of lake sturgeon (*Acipenser fulvescens*), shortnose sturgeon (*A. brevirostrum*), white sturgeon (*A. transmontanus*), green sturgeon (*A. medirostris*), Atlantic sturgeon (*A. oxyrhynchus oxyrhynchus*), gulf sturgeon (*A. oxyrhynchus desotoi*), pallid sturgeon (*Scaphirhynchus albus*), and shovelnose sturgeon (*S. platorhynchus*).

Extraction

DNA was extracted from 0.5–1.0 cm^2 fin samples by the CTAB method of Saghai-Maroof et al. (1984) and Doyle and Doyle (1987) as modified by Grewe et al. (1993). The resultant DNA was highly degraded but most of the DNA was >4 kilobases (kb).

Library screening

Extracted DNA from four lake sturgeon was pooled (6 µg) and digested with Sau3A. The plasmid pUC 18 was digested with *Bam*HI, purified with phenol–chloroform, and precipitated with ethanol. Digested pUC 18 (100 ng) was added to non-size-fractionated sturgeon DNA in 1:2, 1:1, and 2:1 weight ratios (50, 100, or 200 ng of sturgeon DNA, respectively) in a ligation mixture containing DNA, buffer, and 1.5 units of T4 DNA ligase. Ligation took place overnight in a thermal cycler at 16°C.

The ligation mix (1 µL) was added to 50 µL of DH5α cells (GIBCO), put on ice for 30 min, incubated for 45 s in a 42°C water bath, and then placed on ice for 2 min. SOC medium (900 µL) was added and the transformed DH5α cells were incubated for 1 h at 37°C. Each transformation (100 µL) was transferred to agar plates that contained ampicillin and allowed to grow overnight yielding from 12 000 to 180 000 colonies. Agar plates then were innoculated to a concentration of about 500–1000 colonies per plate and incubated overnight.

Magnagraph nylon transfer membranes (MSI) were wetted briefly in water and then layered over each plate for 2 min. Membranes lifts were then placed in 10% SDS for 5 min, transferred to denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 20 min, then placed in a neutralizing solution (1.5 M NaCl, 0.5 M Tris; pH 7) for 15 min, and finally transferred to 2 × SSC for 10 min. The membranes were then baked at 80°C for 1 h.

Membrane lifts were hybridized with tetra- and tri-nucleotide repeat probes supplied in the BIOS oligo scan kit (AAAT, AAAG, AAAC, CCG, AAG, AAT, AAC, and CAG) or made at the Cornell DNA synthesis facility (GATA, GACA and GGAT). AAAT, AAAG, AAAC, CCG, AAG, AAT, AAC, and CAG were used to screen "lift set I." AAAT, AAAG, AAAC, CCG, AAG, GATA, GACA, and GGAT were used to screen "lift set II." Nucleotide repeats were 3′-end labeled with digoxigenin-11-ddUTP using the Genius 5 Oligonucleotide 3′-end labeling kit (Boehringer-Mannheim). Membrane lifts were prehybridized in Hybaid hybridization bottles in 10 mL hybridization solution (0.5 M sodium phosphate (pH 7.2) 0.5 M EDTA, 20% SDS, and 1% BSA) at 58°C overnight. The next day the hybridization solution was removed and fresh solution containing 1–2 pM of each labeled repeat. Lifts were thus incubated overnight at 58°C. Lifts were then washed three times for 15 min each with 2 × SSC and 0.1% SDS (twice at 21°C and once at 58°C) and once with 0.5 × SSC and 0.1% SDS for 15 min at 21°C. The Genius colorimetric detection kit, which uses NBT and X-phosphate (Boehringer-Mannheim), was used to visualize digoxygenin-labeled repeat probes with the following conditions: Genius buffer 1 for 1 min, Genius buffer 2 for 2.5 h, Genius buffer 2 with FAB α digoxygenin (10 µL/100 mL Genius buffer 2) for 30 min, Genius buffer 1 for 15 min, Genius buffer 1 for 15 min, Genius buffer 3 for 2 min, and 10 mL Genius buffer 3 with 45 µL NBT and 35 µL X-phosphate until development was complete (30 min – 12 h).

Secondary screening

Dot blotting directly

Positively hybridizing colonies were picked from plates with a sterile toothpick and transferred into a 13 × 100 mm culture tube with 5 mL Terrific Broth (TB). After growing overnight at 37°C, 2 µL of the culture was dot blotted onto a piece of Schleicher and Schuell Nytran Plus membrane and allowed to dry. Pretreatment and hybridization of this membrane was identical to that procedure used for the original membrane lifts. Colonies that again were positive were streaked onto an agar plate and grown overnight at 37°C. Four separated colonies from each plate were then picked, used to inoculate 1 mL of TB in 1.5 mL microcentrifuge tubes, and incubated overnight in a heat block at 37°C. Two microlitres were dot blotted from each tube and rescreened.

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Secondary plating

Alternatively, colonies in the area of the positively hybridizing colonies were picked and grown overnight. This growth was diluted 1 : 10 000 and 10 µL were innoculated onto agar plates (usually to a concentration of 50–200 colonies per plate). Colonies were lifted, and lifts were hybridized as described above. Membranes were lined up with plates so individual, positive colonies could be picked. Positively hybridizing colonies then were placed in 5 mL TB with ampicillin and grown overnight at 37°C in 50-mL conical centrifuge tubes. Two microlitres of the overnight growth from each tube was dot blotted and rescreened.

Sequencing of clones

Positive clones were innoculated in 5 mL TB and grown overnight. From this growth, 0.5 mL was added to 30% glycerol in TB and frozen to –70°C for future use and 1.5 mL were purified using the Qiagen plasmid mini-kit to obtain 6–20 µg of purified DNA. Twelve microlitres of each clone at ~200 ng/µL was sent to the Cornell Biotechnology Center's Analytical Chemistry and Peptide/DNA Synthesis Facility (ACPSF) along with 10 µL M13 forward primers at 5 µg/mL for sequencing on an ABI 373A automated sequencer.

Primer design, synthesis, and testing

Primers were designed with Generunner and were synthesized at the ACPSF. Primers were first tested with five individual lake sturgeon and subsequently with six lake, four Atlantic, four white, five pallid, four shovelnose, five shortnose, five gulf, and six green sturgeon.

PCR and gel conditions

Amplifications were done in 500-µL tubes with 1 unit *Taq* DNA polymerase (GIBCO), 0.4 µM of each primer, 3–10 ng template DNA (prepared by the CTAB method described above), 100 or 175 µM dNTPs, 1.5–2.5 mM $MgCl_2$ in 50 µL of buffer. Reaction mixes were amplified in a Perkin Elmer 4800 cycler as follows: preheating at 94°C for 3 min, 35 cycles of amplification (denaturing at 94°C for 1 min, annealing at 57°C for 30 s, polymerization (elongation) at 72°C for 30 s), and a final polymerization at 72°C for 5 min.

Amplified products were run on a 3.5% agarose gel (Nusieve GTG : SeaKem LE, 1:1; 27.5 × 22 × 0.6 cm; 1 × TBE) at 150 V for 1 h or on a 4% Metaphor agarose gel (27.5 × 22 × 0.3 cm; 1 × TBE) at 460 V for 1.5 h. A buffer (0.5 × TBE) temperature of 18°C was maintained for the Metaphor gels with a Neslab model RTE100 refrigerated coolant pump, after an initial 7-min loading run at 13°C.

Results

Approximately 61 000 colonies were screened in lift set I yielding 110 positively hybridizing colonies, of which 33 colonies were sequenced that were found positive three times. Among approximately 31 000 colonies screened in lift set II, 71 positive colonies were recognized, of which 24 colonies were sequenced. From the 57 colonies sequenced, (*i*) primers were made for 14 tri- and tetra-meric microsatellite loci, (*ii*) 18 possessed a minisatellite repeat of 125 bp, which also contained two trimeric motif microsatellite loci (B. May, C.C. Krueger, and H.L. Kincaid, unpublished data), (*iii*) five contained multiple clones, (*iv*) two contained poly As, (*v*) six contained microsatellites for which primers could not be effectively designed, (*vi*) one contained a 40-bp repeating sequence too close to the vector, (*vii*) three inserts were identical to other inserts, (*viii*) one had a dimeric repeat motif, and (*ix*) seven had sequencing problems.

Eleven of the 14 microsatellite primer pairs produced an electrophoretically resolvable product when tested on five or more lake sturgeon (Table 1). Four of these loci possessed pure trimer repeat motifs, five had pure tetramer repeats, one had two back-to-back tetramer repeats, and one had a tetramer repeat followed by a trimer repeat in the sequenced clones. The number of repeats in individual clones varied from 6 to 30 and all were uninterrupted perfect repeats. Locus designation, insert size, repeat motif, number of repeats in the clone, primer sequences, GenBank accession numbers, optimal $MgCl_2$ and dNTP concentrations, and allele size in the clone for the 11 loci are listed in Table 1. Three of the 14 primer pairs did not produce a consistently resolvable product.

Of the 11 primer pairs that yielded a product in lake sturgeon, eight of the primers produced an electrophoretically resolvable product in all of the other seven species of sturgeon (Table 1). One primer pair (LS-22) functioned only with lake and green sturgeon; another (LS-23) produced products only in lake, Atlantic, shortnose and green sturgeon; while LS-69 produced a product in all but green sturgeon. Though only four to six individuals of each species were tested, alternate allelic forms were present at 9 of the 11 loci in one or more species. Polymophisms were observed at one locus (LS-68) in all eight species. One locus (LS-58) exhibited a single band identical to the clone for all individuals of all species. Three to eight loci were polymorphic per species. Fifty-six percent of the species–locus combinations that produced amplicons were polymorphic (based on presence of trailing presumptive "heteroduplex" bands, see Discussion) when run on the 3.5% agarose gels.

Discrete bands differing by one or more repeat units were observed when amplified samples were run on Metaphor agarose gels. For example, seven alleles can be observed among two pallid and six shovelnose sturgeon for locus LS-68 (Fig. 1). Each shovelnose sturgeon was heterozygous for a different combination of alleles. While this locus appears to be disomic, other loci clearly displayed four gene doses (Fig. 2). In addition, one or two extra bands with slower mobility were observed in individual heterozygous phenotypes (Figs. 1 and 2).

Discussion

Primer sequence homology in *Acipenser* and *Scaphirhynchus* was observed for 8 of 11 microsatellite loci in eight species. Additionally, variation in microsatellite repeat number may also be conserved as the amplified products of each individual differed in size by the equivalent of <10 repeats among all species examined. Conservation among related taxa of the unique flanking regions around microsatellites has been reported by others. Moore et al. (1991) report that 56% of 48 primer pairs designed for cattle also produced a product in sheep, 6% in horses, and none in humans, indicating that homology of the flanking regions corresponds to relatedness. Complete conservation has been shown for 10 loci for four species within the cat family Felidae (Menotti-Raymond and O'Brien 1995) and for six loci across six species within two turtle families, Cheloniidae and Dermochelyidae (FitzSimmons et al. 1995). These latter studies suggest that a well-developed group of primers in one species may function well in genetic studies of related taxa within families and thus reduce the time and effort required for primer development.

A high level of polymorphism was exhibited at the microsatellite loci resolved, especially considering the few individuals

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Table 1. Microsatellite loci in eight species of sturgeon.

Locus	Insert size (bp)	Repeat motif	Primer	Primer sequence	GenBank accession no.	$MgCl_2$ (mM)	dNTPs (μM)	Clone size (bp)	LS[a] (6[b])	AS (4)	WS (4)	PS (5)	SV (4)	SN (5)	GF (5)	GR (6)
LS-19	267	$(TTG)_9$	LS-19F[c]	CATCTTAGCCGTCTGTGGTAC	U72730	1.5	175	133	p[d]	P	M	P	P	P	P	P
			LS-19R	CAGGTCCCTAATACAATGGC												
LS-22	570	$(AAAT)_6$	LS-22F	TCCACAATCCTGAATAATGAC	U72731	1.5	100	218	P	N	N	N	N	N	N	M
		$(AAG)_{30}$	LS-22R	GCACCATCTAATACGAAATTG												
LS-23	278	$(GTTT)_8$	LS-23F	TGCAGTAGATCATTGGTGG	U72732	1.5	175	162	M	M	N	N	N	M	N	M
			LS-23R	CCTACATCCCACNACGGTAG												
LS-34	448	$(GTT)_{10}$	LS-34F	TACATACCTTCTGCAACG	U72733	2.5	175	137	M	M	M	P	P	P	M	M
			LS-34R	GATCCCTTCTGTTATCAAC												
LS-39	700	$(GTT)_{10}$	LS-39F	TTCTGAAGTTCACACATTG	U72734	2.0	100	129	P	M	M	M	M	P	P	M
			LS-39R	ATGGAGCATTATTGGAAGG												
LS-54	500	$(GATA)_6$	LS-54F	CTCTAGTCTTTGTTGATTACAG	U72735	1.5	175	177	M	P	M	P	P	P	P	M
		$(GACA)_7$	LS-54R	CAAAGGACTTGAAACTAGG												
LS-57	1100	$(GAA)_{29}$	LS-57F	GCTTGGTTGCTAGTTTGC	U72736	1.5	100	206	P	M	P	P	P	P	M	P
			LS-57R	GTACAGATGAGACCAGAGGC												
LS-58	209	$(GATA)_{20}$	LS-58F	TCGGTTGAGTGATTGGTATC	U72737	1.5	100	198	M	M	M	M	M	M	M	M
			LS-58R	CTGAAAGGTGAATCTACTCCC												
LS-62	190	$(GACA)_7$	LS-62F	GATCAGGAGGGCAGAGNAAC	U72738	2.5	175	85	P	P	P	P	P	P	M	P
			LS-62R	CCCTGGATTTGAATTAACAG												
LS-68	1500	$(GATA)_{13}$	LS-68F	TTATTGCATGGTGTAGCTAAAC	U72739	1.5	100	120	P	P	P	P	P	P	P	P
			LS-68R	AGCCCAACACAGACAATATC												
LS-69	300	$(TATC)_{13}$	LS-69F	ATCTGAATTGANTTCGTG	U72740	2.0	100	206	P	M	M	M	M	P	P	N
			LS-69R	TTGGATACTGTGTTCCAAC												

[a] LS, lake sturgeon; AS, Atlantic sturgeon; WS, white sturgeon; PS, pallid sturgeon; SV, shovelnose sturgeon; SN, shortnose sturgeon; GF, gulf sturgeon; GR, green sturgeon.
[b] Number of each species examined.
[c] F, forward primer; R, reverse primer (5′ to 3′).
[d] M, monomophic; P, polymorphic; N, no activity.

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Fig. 1. Ethidium bromide stained 4% Metaphor agarose gel of LS-68 amplification products of six shovelnose and two pallid (the two 66 individuals) sturgeon individuals. Presumptive numerical genotypes for a single disomic locus (e.g., a 34 indicates an individual heterozygous for alleles 3 and 4) are above each individual and labeled arrows indicate the alleles that produce particular bands. In heterozygous phenotypes the faster (lower molecular weight) bands are the real bands while the slower bands are presumptive heteroduplex bands (marked by an h) composed of complementary strands of the two component alleles. Specific alleles appear to migrate to positions as follows: 1, 116 bp; 2, 120 bp; 3, 124 bp; 4, 128 bp; 5, 132 bp; 6, 136 bp; and 7, 140 bp, as expected for a microsatellite with a motif of 4 bp (GATA).

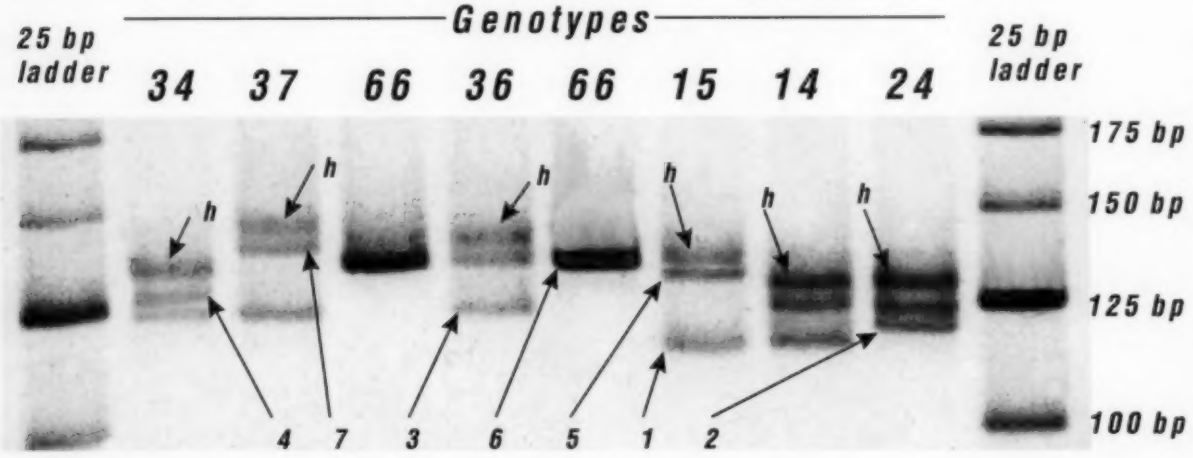
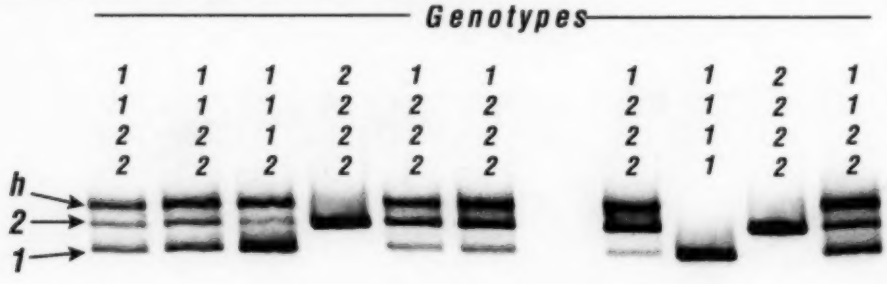


Fig. 2. Ethidium bromide stained 4% Metaphor agarose gel of Atlantic sturgeon amplification products from 10 progeny from a family segregating for LS-19. Bands are coded on left by the alleles 1 and 2 that they represent. Note the extra single slower migrating artifactual heteroduplex band (designated by an h) in the three-banded heterozygous phenotypes. Presumptive genotypes are listed above each respective phenotype, clearly showing the ability to differentiate the three different types of heterozygotes in a four gene dose system with two alleles. The two parental individuals for this family were both 1122 genotypes. Allele 1 has apparent mobility of 131 bp and allele 2 of 143 bp, suggesting a difference of four TTG repeats between these two alleles.



examined and the gel conditions used. Polymorphic loci were detected using 3.5% agarose gels and analyzing samples from four to six individuals of each species, often taken from a single location or population. Only a few locus–species combinations were analyzed on Metaphor agarose gels to demonstrate the resolving power of this medium (Figs. 1 and 2). All microsatellite loci except two (LS-23 and LS-58) contained one or more alternate alleles. The use of higher resolving Metaphor agarose gels and the inclusion of more individuals from different populations likely will reveal new alleles at the polymorphic loci as well as polymorphisms in systems reported here as monomorphic in a particular species. Further, alterations of amplification conditions may permit amplification of those species–locus combinations that failed to amplify for us. LS-58 was fixed for the same allele in all species tested. Possibly some evolutionary constraint exists for this microsatellite locus that prevents structural change (e.g., it may lie in a transcribed but nontranslated region for a structural gene). In addition, these 4% Metaphor gels clearly permit scoring of gene dosage in heterozygous individuals, a condition of the polyploid ancestry of these fish.

Heterozygous individuals were characterized by the presence of bands that were slower than the allelic bands. Presumably these bands were the result of heteroduplex molecules formed as combinations of complementary strands (from the two different alleles) reannealing after denaturation without amplification. For most loci we saw only one slower band (see Figs. 1 and 2), but occassionally two bands were observed. The presence of one versus two bands may be caused by a difference in annealing and secondary folding (and hence gel

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migration rate) between the two different types of heteroduplex molecules.

The microsatellite loci described here provide a much needed group of genetic markers that are detectable with nonlethal sampling (blood, barbel, or fin). Small amounts of tissue may be sampled from individuals of sturgeon populations that are threatened or endangered without causing the death of the fish. Variation at microsatellite loci can be useful in many types of biological studies such as parentage testing, mapping, and population structure (O'Reilly and Wright 1995). These loci are particularly useful because they are codominant in the phenotypic expression of their alleles, exhibit more alleles segregating per locus than allozymes, and have higher mutation rates (Weissenbach et al. 1992) than allozymes. These primers detected genetic variation at microsatellite loci in both *Acipenser* and *Scaphirhynchus*, and thus these primers likely will perform well in sturgeon species worldwide for the analysis of genetic variation at microsatellite loci.

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Genetic variation of microsatellite and mitochondrial DNA markers in broad whitefish (*Coregonus nasus*) in the Colville and Sagavanirktok rivers in northern Alaska

John C. Patton, Benny J. Gallaway, Robert G. Fechhelm, and Matthew A. Cronin

Abstract: There has been concern that a causeway leading to oil production facilities in the Alaskan Beaufort Sea could affect the extent of emigration from, and immigration into, a population of broad whitefish (*Coregonus nasus*) in the Sagavanirktok River. To assess this, we analyzed the genetic relationships of the broad whitefish populations in the Sagavanirktok River, and the nearest adjacent population, in the Colville River. Three microsatellite loci from the nuclear genome, and the NADH-1 gene of mitochondrial DNA (mtDNA), were analyzed. Diploid genotypes were determined with PCR amplification of the microsatellite loci, and mtDNA genotypes were identified with PCR amplification followed by sequencing of 798 nucleotides. Several alleles were identified at each locus and both populations had high levels of genetic variation. There is significant differentiation of the Sagavanirktok River and Colville River broad whitefish stocks for the three microsatellite loci (F_{ST} = 0.031) but not mtDNA ($F_{ST} < 0.001$). Possible explanations for the lower level of differentiation of mtDNA than microsatellites include female-mediated gene flow between populations, skewed sex ratios, natural selection, or mutation. The results indicate that there is limited gene flow between the Colville and Sagavanirktok rivers, which represent semi-isolated spawning populations.

Résumé : On s'est inquiété du fait qu'un pont-jetée qui mène aux installations de production de pétrole dans la mer de Beaufort du côté de l'Alaska pourrait avoir un effet sur l'importance de l'émigration et l'immigration touchant une population de corégones tschir (*Coregonus nasus*) de la rivière Sagavanirktok. Pour évaluer cette situation, nous avons analysé les relations génétiques des populations de corégones tschir dans la rivière Sagavanirktok et dans la population adjacente la plus rapprochée, celle de la rivière Colville. Trois loci de microsatellites du génome nucléaire, et le gène NADH-1 de l'ADN mitochondrial (ADNmt), ont été analysés. Les génotypes diploïdes ont été déterminés par amplification (PCR) des loci de microsatellites et les génotypes d'ADNmt ont été identifiés par PCR et séquençage de 798 nucléotides. Plusieurs allèles ont été identifiés dans chaque locus et les deux populations présentaient un haut degré de variation génétique. On observe une différenciation importante des stocks de corégones tschir de la rivière Sagavanirktok et de la rivière Colville en ce qui a trait à ces trois loci de microsatellites (F_{ST} = 0,031), mais non pour l'ADNmt ($F_{ST} < 0,001$). Parmi les explications possibles du degré plus faible de différenciation dans le cas de l'ADNmt que dans celui des microsatellites figurent le flux génique médié par les femelles entre les populations, des sex-ratios biaisés, la sélection naturelle et les mutations. Les résultats indiquent qu'il y a un flux génétique limité entre les stocks des rivières Colville et Sagavanirktok, qui représentent des populations de reproduction semi-isolées.
[Traduit par la Rédaction]

Introduction

Since the early 1980s, summer fisheries research and monitoring programs have been conducted in the Prudhoe Bay region of Alaska in conjunction with oil exploration and development activities along the Beaufort Sea Coast. The Endicott Development Causeway was constructed in 1985 in the delta of the Sagavanirktok River to provide access to offshore oil and gas fields in the Beaufort Sea (Fig. 1). One of the main issues identified in the project's environmental impact statement (EIS) was the possible effect that the causeway might have on broad whitefish (*Coregonus nasus*), particularly the population that spawns in the Sagavanirktok River (Gallaway et al. 1991). There was concern that the Endicott Causeway might adversely affect broad whitefish growth, either by limiting access to suitable coastal feeding habitat or by altering the hydrographic character of that habitat. The broad whitefish is an abundant amphidromous species that inhabits several of the rivers on the North Slope of the Brooks Range in Alaska, and it is an important component of Native subsistence fisheries.

Ecological studies between 1982 and 1994 indicated that the broad whitefish in the Sagavanirktok River fluctuate in numbers and age structure. There is evidence that changes in

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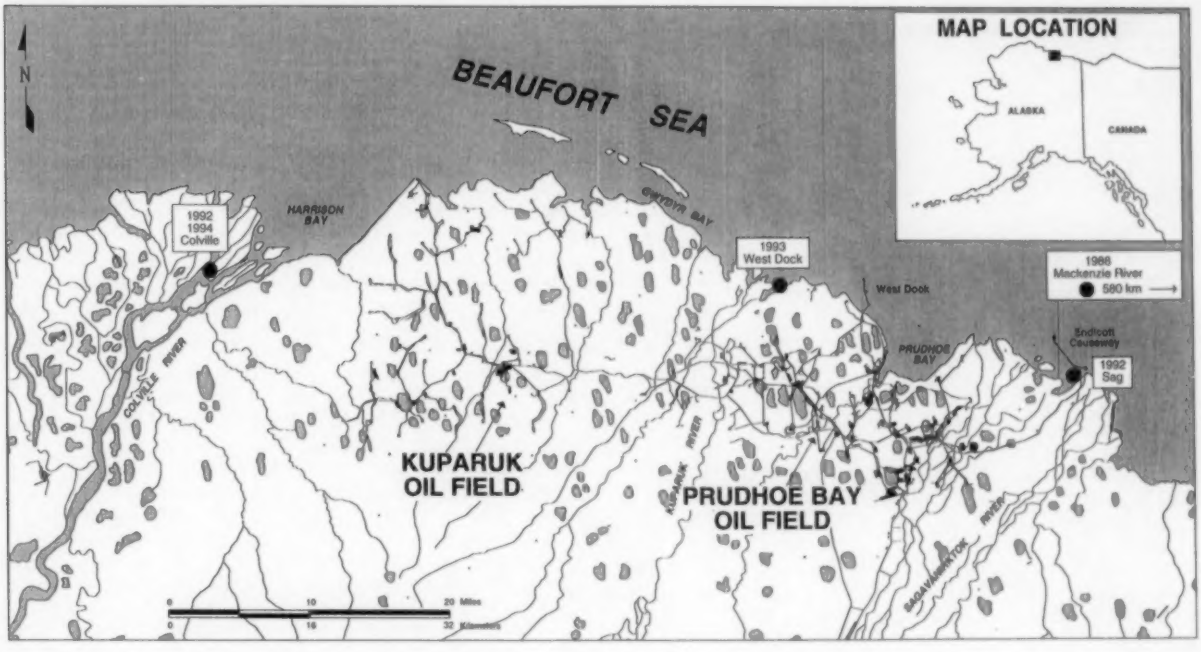
Fig. 1. Map of study area showing locations and years of sampling.



population numbers are caused by natural density-dependent factors, such as limited overwintering habitat in the Sagavanirktok River, and not causeway effects (Gallaway et al. 1997). However, another cause of changes in population numbers could be immigration into, or emigration out of, the Sagavanirktok River. The objective of this study was to assess the likelihood that immigration or emigration could contribute to changes in the population of broad whitefish in the Sagavanirktok River. To achieve this, we quantified molecular genetic differentiation of the broad whitefish in the Sagavanirktok River and the Colville River. The Colville River, 95 km to the west, contains the closest population of broad whitefish that could exchange individuals with the Sagavanirktok River population (Fig. 1).

Molecular genetics methods have been used extensively to quantify differentiation and infer gene flow among fish populations, including Pacific salmon (e.g., Waples and Teel 1990; Cronin et al. 1993; Adams et al. 1994; Bickham et al. 1995; Adkison 1995; Taylor et al. 1996; Scribner et al. 1996) and coregonids (Bickham et al. 1989; Bernatchez and Dodson 1990, 1991; Shields et al. 1990). We focused on maternally inherited mitochondrial DNA (mtDNA) and biparentally inherited short tandem repeat nuclear DNA (i.e., microsatellites). These genetic markers have shown considerable intraspecific variation useful in quantifying population genetic structure (e.g., Cronin et al. 1993; Brooker et al. 1994; Nielson et al. 1994; Scribner et al. 1994, 1996; Fitzsimmons et al. 1995; McConnell et al. 1995; Bernatchez et al. 1996). The use of mtDNA in tandem with nuclear genes has been particularly informative for assessing population genetic structure (e.g., Powell 1983; Takahata and Slatkin 1984; DeSalle et al. 1987; Cronin et al. 1991; Forbes and Allendorf 1991; Bernatchez and Osinov 1995; Taylor et al. 1996).

Materials and methods

Sample collection

Broad whitefish were collected from several areas within the Sagavanirktok River Delta during summer 1992 (Fig. 1). Because younger age cohorts are readily distinguishable within the length–frequency profiles of the overall population (Fechhelm et al. 1992; Griffiths et al. 1992), we were able to determine that samples included young-of-the-year (59–80 mm), 1-year-old (81–100 mm), and 2-year-old (101–180 mm) fish. Small broad whitefish are typically restricted to river deltas and lagoons with relatively low salinity (Morrow 1980; Craig 1989), and thus our assumption was that these younger age cohorts were from the Sagavanirktok River spawning stock.

In 1993, young-of-the-year broad whitefish were collected from a sampling site west of West Dock (Fig. 1) in the Kuparuk River Delta. Several lines of evidence suggested that these fish were of Colville River origin. First, small broad whitefish seldom occur in the Kuparuk River Delta in appreciable numbers, a trend which suggests that the Kuparuk River does not support a spawning stock (LGL Alaska Research Associates, Inc. 1995). Young fish collected in the Kuparuk Delta, therefore, are most likely of either Sagavanirktok River or Colville River origin. However, the appearance of the young-of-the-year broad whitefish late in 1993 occurred simultaneously with the appearance of large numbers of small (<100 mm) least cisco (*Coregonus sardinella*). This size-class of least cisco is rarely collected in the Prudhoe Bay area. This collection was made immediately after 2 days of strong westerly winds. Because least cisco spawn in the Colville River and not in the Sagavanirktok or Kuparuk rivers (Craig 1984), the young least cisco must have moved into the region from the west (i.e., the Colville River). The coastal movement of small least cisco between the Colville River and Prudhoe Bay is strongly correlated with wind-driven coastal currents (Fechhelm et al. 1994), and we hypothesized that the small broad whitefish and least cisco in the Kuparuk Delta were transported eastward from the Colville River during the late-summer period of strong west winds.

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Table 1. Nucleotides that occurred at variable positions in the ND1 mtDNA gene of broad whitefish.

MtDNA genotype (allele)	Nucleotide position					
	153	195	293	501	504	585
A	A	G	C	T	A	A
B	G	G	C	C	A	A
C	G	A	C	C	A	A
D	A	G	C	T	A	G
E	G	A	C	C	G	A
F	G	G	T	C	A	A

We also collected twenty adult broad whitefish (>250 mm) in fall 1994 from commercial and subsistence fisheries in the Colville River. In addition, six young-of-the-year fish were collected in fyke nets in the Colville Delta in 1992 and were combined with the 1994 adults into one Colville River sample. Three additional broad whitefish, collected during the summer of 1988 from the Mackenzie River, Canada (500 km east of the Sagavanirktok River), were also analyzed to provide a broader geographic perspective. All samples were frozen or preserved in buffer for shipment to the LGL Ecological Research Associates, Inc., (LGL) laboratory in Texas.

Laboratory methods

DNA was extracted from muscle tissue using procedures which include incubation in SDS and proteinase K, followed by phenol–chloroform extractions (Sambrook et al. 1989; Cronin et al. 1993), and the polymerase chain reaction (PCR) was used to amplify the DNA fragments of interest (Saiki et al. 1988).

Mitochondrial DNA analysis

The NADH–1 (ND1) region was amplified with oligonucleotide primers LGL290: 5′-GGCCTAAGCCCTTTTTCTCA–3′ and LGL560: 5′-GGC(A/G)GCTTTTGGTTATTAG–3′. The LGL 290 primer is positioned in the leucine tRNA between the 16S and ND1 genes at human mtDNA nucleotide positions 3238–3257. The LGL 560 primer is located in the 3′ end of the ND1 gene at human mtDNA positions 4100–4081 (Anderson et al. 1981). Each PCR reaction was composed of 0.1–0.2 μg of genomic DNA; 5 μL of 10× buffer (0.1 M Tris-HCl, pH 8.7, 0.5 M KCl, 0.025 M $MgCl_2$, 0.5 $\mu g \cdot \mu L^{-1}$ bovine serum albumin); 5 μL of dNTP mix (2 mM each of dATP, dTTP, dCTP, and dGTP in 10 mM Tris-HCl, pH 7.9); 5 μL of a 2 $pM \cdot \mu L^{-1}$ solution of both primers; 1.5 units of *Taq* polymerase; and enough distilled, deionized water to bring the reaction volume to 50 μL. The thermal cycler profile was 32 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 40 s, extension at 70°C for 90 s, plus an autoextension of 2 s per cycle. Two microlitres of each amplification were screened on a 0.8% submersed agarose gel using TEA buffer (Sambrook et al. 1989), stained with ethidium bromide, and photographed to determine the quality and concentration of product. The remaining product was prepared for sequencing by adding 2 mL of TE buffer to the product in a Centricon 100 tube (Amicon, Inc., Beverly, Mass.) and centrifuging the sample as recommended by the manufacturer.

Sequence reactions were performed using the Applied Biosystems (ABI, Foster City, Calif.) *Taq* Dye Deoxy™ terminator cycle sequencing kit. The recommended premix was added to approximately 100 ng of Centricon-purified template DNA, 3.2 pM of sequencing primer, and water to bring the final volume to 20 μL. The reaction mix was overlaid with one drop of mineral oil. Sequence reaction parameters were 96°C for 30 s, 50°C for 15 s, and 60°C for 2 min for 25 cycles. Both amplification primers were used for sequencing, one from each direction, to obtain the complete sequence. Samples were run on an ABI 373A autosequencer (ABI, Foster City, Calif.). The chromatograms produced were analyzed and aligned using the SeqEd™ (ABI, Foster City, Calif.) sequence editing software. Mitochondrial DNA genotypes were identified from the DNA sequences. Mitochondrial DNA is maternally inherited without genetic recombination in the egg cytoplasm and generally occurs as a single genotype in individuals, although multiple genotypes within individuals may occur (Bermingham et al. 1986).

Microsatellite loci

Three microsatellite loci were used in this study. Microsatellites are stretches of DNA consisting of di-, tri-, or tetra-nucleotide repeats. They are common in eukaryotic nuclear genomes and are often highly variable (Haymer 1994). Because of these properties, microsatellites are increasingly being used in studies of population genetic structure (e.g., Scribner et al. 1996). Primers for two loci, LGL BWF1 and LGL BWF2, were developed by LGL. The PuPuPy microsatellite locus, originally developed by Morris et al. (1996) for rainbow trout (*Onchorhynchus mykiss*), was redesigned from sequences kindly provided by J. Wright (Department of Biology, Dalhousie University, Halifax, N.S.).

The LGL BWF1 and LGL BWF2 loci were developed by cutting genomic broad whitefish DNA with *Apo*I and cloning these genomic fragments into the *Eco*RI site of the Bluescript® plasmid vector (Stratagene, La Jolla, Calif.). Transformation reactions were completed using Epicurian Coli® ultracompetent cells (Stratagene, La Jolla, Calif.) following the protocol supplied by the manufacturer. Approximately 2000 recombinant bacteria were screened using nonradioactive colorometric protocols (Genius System, Boehringer Mannheim, Indianapolis, Ind.) with an end-labeled $(CA)_{11}$ oligonucleotide probe. Of the eight positive colonies obtained, three had inserts containing more than 12 CA repeats. The inserts were sequenced and primers were designed in the sequence flanking the repeat for two of the positive clones. The LGL BWF1 locus contains two repeats in the sequence $(GA)_{16}$ $(N)_{95}$ $(TG)_{13}$, while LGL BWF2 contains a $(CA)_{25}$ repeat.

The primer sequences are as follows:

LGL BWF1

5′-TACAGAGAAATACACACAACGCATCAA-3′

5′-GAGAGGTTCCATTACTGAGCAC-3′

LGL BWF2

5′-CGGATACATCGGCAACCTCTG-3′

5′-AGACAGTCCCCAATGAGAAAA-3′

LGL PuPuPy

5′-GGGGAGCATGCAGCGGATGTA-3′

5′-GCCGGTGGGTAAAGAATGCAGC-3′

One primer of each of the three microsatellite loci was labeled with one of the fluorochromes used in the ABI GeneScan™ system (ABI, Foster City, Calif.). These loci were amplified with the same PCR conditions as for the mtDNA ND1 fragment, except the extension time was 45 s and the autoextension was one second per cycle. The three microsatellite loci for each fish were run simultaneously in a single lane on the ABI 373A (ABI, Foster City, Calif.) autosequencer. Results were compiled and analyzed with GeneScan™ and Genotyper™ 1.1 software (ABI, Foster City, Calif.).

Data analysis

Each sampling group (year 0, year 1, and year 2, Sagavanirktok River, West Dock, Colville) were analyzed for Hardy–Weinberg equilibrium with goodness of fit chi-square tests. The extent of genetic differentiation among year-classes and sampling locations was assessed with estimation of F_{ST} and heterogeneity chi-square analyses of allelic variation. F_{ST}, a standardized variance of allele frequencies, is a commonly used index of population genetic subdivision (Wright 1978). The chi-square tests were done with the Monte Carlo simulation method described by Roff and Bentzen (1989, 1992). In this method a standard chi-square value (e.g., Workman and Niswander 1970) is

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Table 2. Microsatellite and mtDNA allele frequencies for broad whitefish from all sampling locations.

	Sampling group							
	Sagavanirktok River							
Locus and allele	Year 0	Year 1	Year 2	Pooled	West Dock	Colville	West Dock–Colville pooled	Mackenzie
MtDNA								
n	34	46	53	133	25	22	47	3
A	0.235	0.261	0.340	0.286	0.320	0.409	0.362	0.333
B	0.735	0.500	0.415	0.526	0.520	0.409	0.468	0.333
C	0.000	0.239	0.245	0.180	0.160	0.136	0.149	0.000
D	0.000	0.000	0.000	0.000	0.000	0.045	0.021	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333
F	0.029	0.000	0.000	0.008	0.000	0.000	0.000	0.000
BWF1								
n	32	49	52	133	24	26	50	3
A	0.000	0.000	0.000	0.000	0.021	0.000	0.010	0.000
B	0.813	0.786	0.856	0.820	0.688	0.750	0.720	0.333
C	0.000	0.000	0.000	0.000	0.021	0.038	0.030	0.167
D	0.094	0.082	0.038	0.068	0.063	0.019	0.040	0.167
E	0.094	0.133	0.106	0.113	0.208	0.192	0.200	0.333
BWF2								
n	32	49	52	133	24	26	50	3
A	0.016	0.000	0.019	0.011	0.021	0.058	0.040	0.000
B	0.000	0.000	0.000	0.000	0.021	0.058	0.040	0.000
C	0.109	0.122	0.221	0.158	0.313	0.288	0.300	0.333
D	0.063	0.041	0.087	0.064	0.042	0.019	0.030	0.000
E	0.063	0.020	0.019	0.030	0.042	0.000	0.020	0.000
F	0.000	0.000	0.000	0.000	0.021	0.038	0.030	0.000
G	0.000	0.000	0.010	0.004	0.021	0.019	0.020	0.167
H	0.547	0.429	0.442	0.462	0.021	0.077	0.050	0.500
I	0.047	0.276	0.106	0.154	0.208	0.173	0.190	0.000
J	0.000	0.000	0.000	0.000	0.021	0.019	0.020	0.000
K	0.125	0.112	0.096	0.109	0.208	0.212	0.210	0.000
L	0.031	0.000	0.000	0.008	0.000	0.000	0.000	0.000
M	0.000	0.000	0.000	0.000	0.063	0.038	0.050	0.000
PuPuPy								
n	32	49	52	133	24	26	50	3
A	0.000	0.000	0.000	0.000	0.021	0.000	0.010	0.000
B	0.000	0.000	0.000	0.000	0.021	0.000	0.010	0.167
C	0.047	0.031	0.000	0.023	0.021	0.000	0.010	0.000
D	0.250	0.224	0.240	0.237	0.000	0.038	0.020	0.000
E	0.000	0.102	0.058	0.060	0.125	0.173	0.150	0.167
F	0.125	0.071	0.067	0.083	0.208	0.192	0.200	0.000
G	0.188	0.204	0.212	0.203	0.188	0.135	0.160	0.333
H	0.125	0.102	0.192	0.143	0.083	0.154	0.120	0.000
I	0.109	0.061	0.067	0.075	0.063	0.173	0.120	0.167
J	0.047	0.051	0.067	0.056	0.063	0.019	0.040	0.000
K	0.047	0.031	0.010	0.026	0.063	0.000	0.030	0.167
L	0.063	0.122	0.087	0.094	0.125	0.058	0.090	0.000
M	0.000	0.000	0.000	0.000	0.021	0.038	0.030	0.000
N	0.000	0.000	0.000	0.000	0.000	0.019	0.010	0.000

calculated from the actual allele frequencies of the groups compared. Repeated randomizations of the allele frequencies are made, and chi-square values for the randomized data are calculated. This allows calculation of the probability of obtaining a chi-square value as large as that for the original data by chance alone. This method is useful when sample sizes are small or, as in our case, when there are many rare alleles. We conducted this analysis with 1000 randomizations of the original data. We also calculated Cavalli-Sforza and Edwards (1967) chord genetic distances among populations, and conducted cluster analyses of them using the neighbor-joining (NJ) method (Saitou and Nei 1987). Because individual loci may reflect different patterns of variation (Slatkin 1987; Pamilo and Nei 1988), we analyzed each locus individually as well as all loci combined. For analyses of mtDNA, sample sizes were divided by two to correct for the functional haploidy of mtDNA (Birky et al. 1983; Cronin et al. 1991). F_{ST}, goodness of fit chi-square, and genetic distances were calculated with the BIOSYS-1 Release 1.7 computer program (Swofford and Selander 1981), the heterogeneity chi-square analyses were done with the

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Table 3. Measures of genetic variability at three microsatellite loci in broad whitefish.

Population	*n*	No. of alleles per locus	Heterozygosity[a]
Year-0 Sagavanirktok	32	6.7 (1.9)	0.594 (0.183)
Year-1 Sagavanirktok	49	6.3 (2.0)	0.605 (0.120)
Year-2 Sagavanirktok	52	6.7 (1.9)	0.609 (0.185)
Colville	26	8.3 (2.2)	0.705 (0.161)
West Dock	24	9.7 (2.3)	0.722 (0.111)
Mackenzie	3	4.0 (0.6)	1.000 (0.000)

Note: Values are means with SEs given in parentheses.
[a]Direct-count heterozygosity of microsatellite genotypes.

MONTE program in the REAP 4.0 computer program package (McElroy et al. 1992), and the NJ analyses were done with the MEGA computer program (Kumar et al. 1993).

Our analysis consisted of four separate comparisons of (1) the three Sagavanirktok River (Sag) age-classes (year 0, year 1, and year 2); (2) the West Dock and Colville samples; (3) a pooled Sagavanirktok River population (three age-classes combined) and a pooled Colville and West Dock population; and (4) all five Alaskan sampling groups (three Sagavanirktok year-classes, West Dock, Colville). Because of the small sample size for the Mackenzie River ($N = 3$), we did not include this group in the comparative analysis.

Results

Mitochondrial DNA sequences, including 798 nucleotides, were generated for each of 183 fish. Variable nucleotides were identified at six positions and occurred as six genotypes (= alleles; Table 1). The complete DNA sequences are available from the senior author. Three of the genotypes (D, E, and F) were relatively rare and each occurred in one fish. Genotypes A, B, and C were relatively common across sampling groups, ranging from 0.000 to 0.735 in frequency (Table 2).

Genotypes at the three microsatellite loci were identified for 186 fish. There was considerable genetic variation at the microsatellite loci with five alleles at the BWF1 locus, 13 alleles at the BWF2 locus, and 14 alleles at the PuPuPy locus (Table 2). The mean number of alleles per locus ranged from 6.3–9.7 (excluding the Mackenzie sample) (Table 3). Mean heterozygosity (i.e., the proportion of heterozygous loci in individual fish) ranged from 0.594 to 0.722. The number of alleles per locus and heterozygosity values were higher in the Colville and West Dock samples than in the Sagavanirktok River samples.

Each of the five Alaskan sample groups (three Sagavanirktok River age groups, West Dock, and Colville) was analyzed for Hardy–Weinberg equilibrium for the three microsatellite loci with goodness of fit chi-square tests. Of the 15 tests performed, one showed significant deviation from Hardy–Weinberg equilibrium. This was the BWF2 locus for the year-1 Sagavanirktok sample, in which there were (with pooling of rare alleles) 24 heterozygotes expected, and only 16 heterozygotes observed ($\chi^2 = 5.44$, df = 1, $P = 0.020$).

Analyses of genetic differentiation included comparisons among the three age-classes in the Sagavanirktok River, between the Colville and West Dock samples, among all five Alaskan sampling groups, and between the pooled Sagavanirktok River samples and the pooled Colville – West Dock samples (Table 4). There were varying levels of genetic differentiation depending on the locus and the groups compared. In Table 4 we include the heterogeneity chi-square values and probabilities for the actual allele frequencies along with the ranges of chi-square values and probabilities calculated with the Monte Carlo simulations. The probabilities associated with the actual allele frequencies and the simulations indicate essentially the same degree of differentiation for each set of groups compared. Because of the small sample size, we did not compare the Mackenzie River population with the Alaskan populations. However, many of the same mtDNA and microsatellite alleles are present in both geographic areas, suggesting there is not a major genetic division between them.

Among the three age-classes within the Sagavanirktok River there was relatively high differentiation of mtDNA compared with two of the microsatellite loci (BWF1 and PuPuPy). The F_{ST} value was higher for mtDNA than for these two microsatellites, and the chi-square value was significant for mtDNA (Table 4). This was due primarily to mtDNA alleles B and C, which differ considerably in frequency in the year-0 fish compared to the year-1 and year-2 fish. There was also a significant chi-square value for the BWF2 locus. The C, H, and I alleles at this locus varied in frequency by 0.11–0.23 among the age-classes (Tables 2 and 4).

Comparison of the Colville and West Dock samples indicate very low F_{ST}s and nonsignificant chi-square values for all four loci. This supports the contention that the West Dock sample is composed of fish from the Colville spawning stock.

The chi-square values indicate that there is significant differentiation of all four loci when the five Alaska sampling groups are treated independently. The F_{ST} estimates for the microsatellite loci among these five groups are higher than in the comparisons of the three Sagavanirktok River age-classes, or between the Colville and West Dock samples. The F_{ST} for mtDNA among these five groups was higher than in the comparison of Colville and West Dock but lower than in the comparison of the three Sagavanirktok River age-classes.

We also pooled samples into a Sagavanirktok River population and a Colville – West Dock population. Although there were significant differences in allele frequencies for two loci among the three Sagavanirktok age-classes (Table 4), we felt justified in pooling them because the small size of these fish supports their origin in the Sagavanirktok River (see the description of sample collection). The comparison of the pooled Sagavanirktok River samples and the pooled Colville – West Dock samples showed relatively high F_{ST} values and significant chi-square values for all three microsatellites. The degree of differentiation is not extreme, as many alleles have generally similar frequencies. However, six microsatellite alleles differed in frequency by ≥0.10 between the two populations: BWF1 allele B; BWF2 alleles C, H, and K; and PuPuPy alleles D and F. In contrast, there is very little differentiation of mtDNA allele frequencies between the pooled samples, as indicated by low F_{ST} and nonsignificant chi-square values (Table 4). No mtDNA allele differed in frequency by >0.08 between the populations. These results indicate the Sagavanirktok River and Colville River broad whitefish stocks are genetically differentiated at nuclear loci but not mtDNA.

In addition to the F_{ST} and chi-square analyses we calculated genetic distances among the five Alaskan sampling groups (Table 5). The NJ analysis of the genetic distances resulted in six dendrograms: one for each of the three microsatellites, one

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Table 4. Results of tests for heterogeneity among broad whitefish sampling groups.

Sampling groups compared and locus	F_{ST}	Chi-square$_o$[a]	Probability	Monte Carlo range of chi-square$_r$[b]	Monte Carlo probability[c]
Three sampling groups: Sagavanirktok year 0, year 1, and year 2					
BWF1	0.000	3.14	0.5350	0.07–15.85	0.5310
BWF2	0.013	36.00	0.0029[d]	5.93–38.74	0.0010[d]
PuPuPy	0.002	21.64	0.2483	4.35–43.40	0.2420
Mean, 3 microsatellites	0.006				
mtDNA	0.041	16.23	0.0122[d]	1.75–24.46	0.0040[d]
Mean microsatellites and mtDNA	0.014				
Two sampling groups: Colville and West Dock					
BWF1	0.000	2.68	0.6131	1.23–14.40	0.6150
BWF2	0.000	6.62	0.8292	1.54–30.45	0.8730
PuPuPy	0.002	16.38	0.2291	4.89–28.95	0.2060
Mean, 3 microsatellites	0.001				
mtDNA	0.000	1.74	0.5789	1.01–13.73	0.6490
Mean microsatellites and mtDNA	0.001				
Five sampling groups: Sagavanirktok year 0, year 1, year 2, West Dock, and Colville					
BWF1	0.002	25.71	0.0583	5.95–37.51	0.0400[d]
BWF2	0.065	139.19	<0.0001[d]	27.63–85.58	<0.0001[d]
PuPuPy	0.017	98.65	0.0001[d]	27.24–87.56	<0.0001[d]
Mean, 3 microsatellites	0.033				
mtDNA	0.019	26.96	0.0392[d]	6.45–36.60	0.0290[d]
Mean microsatellites and mtDNA	0.026				
Two sampling groups: three Sagavanirktok year 0, year 1, year 2 pooled; West Dock – Colville pooled					
BWF1	0.006	16.52	0.0024[d]	0.44–15.36	<0.0001[d]
BWF2	0.058	94.06	<0.0001[d]	3.08–31.28	<0.0001[d]
PuPuPy	0.017	54.52	<0.0001[d]	2.39–33.85	<0.0001[d]
Mean, 3 microsatellites	0.031				
mtDNA	0.000	4.27	0.3734	0.75–15.47	0.3660
Mean microsatellites and mtDNA	0.020				

[a]Chi-square$_o$, chi-square value from chi-square contingency analysis (without pooling).
[b]Chi-square$_r$, chi-square range of values obtained from 1000 randomizations of the allele frequency data.
[c]The probability is the proportion of 1000 randomizations where chi-square$_r$ ≥ chi-square$_o$.
[d]Statistically significant.

for mtDNA, one for the three microsatellites combined, and one for all four loci combined (Fig. 2). For the three microsatellite loci, individually or combined, and the three microsatellites and mtDNA combined, the three age-classes of the Sagavanirktok River clustered together, and the Colville and West Dock clustered together. As indicated by the F_{ST} and chi-square analyses, the year-classes in the Sagavanirktok River are considerably differentiated for mtDNA. This resulted in the year-0 Sagavanirktok fish clustered apart from the other four sampling groups in the mtDNA dendrogram (Fig. 2E).

Table 5. Cavalli-Sforza and Edwards (1967) chord genetic distances among sampling groups for three microsatellite loci (below diagonal) and mtDNA (above diagonal).

	Sag year 0	Sag year 1	Sag year 2	West Dock	Colville
Sag year 0	—	0.344	0.374	0.287	0.338
Sag year 1	0.201	—	0.106	0.070	0.181
Sag year 2	0.184	0.149	—	0.124	0.183
West Dock	0.404	0.345	0.359	—	0.159
Colville	0.390	0.333	0.312	0.228	—

Discussion

Our results suggest the following key points: there is considerable genetic variation of microsatellites and mtDNA in broad whitefish of the Sagavanirktok and Colville rivers; there is genetic heterogeneity among year-classes of the Sagavanirktok River stock; the West Dock samples are derived from the Colville stock; and there is considerable differentiation of the Sagavanirktok River and Colville River stocks for the microsatellite loci, but not for mtDNA.

As observed in other studies, variation (in terms of numbers of alleles and heterozygosity) is high for the microsatellite loci we analyzed (Bentzen et al. 1991; Brooker et al. 1994; Scribner et al. 1994, 1996; Wright and Bentzen 1995). We observed more alleles and heterozygosity in the Colville River than the Sagavanirktok River. One possible explanation for this is that the Colville River is about four times larger with more overwintering habitat than the Sagavanirktok River (Selkregg 1974). A larger effective population size may have contributed to the higher level of genetic variation observed in the Colville River broad whitefish.

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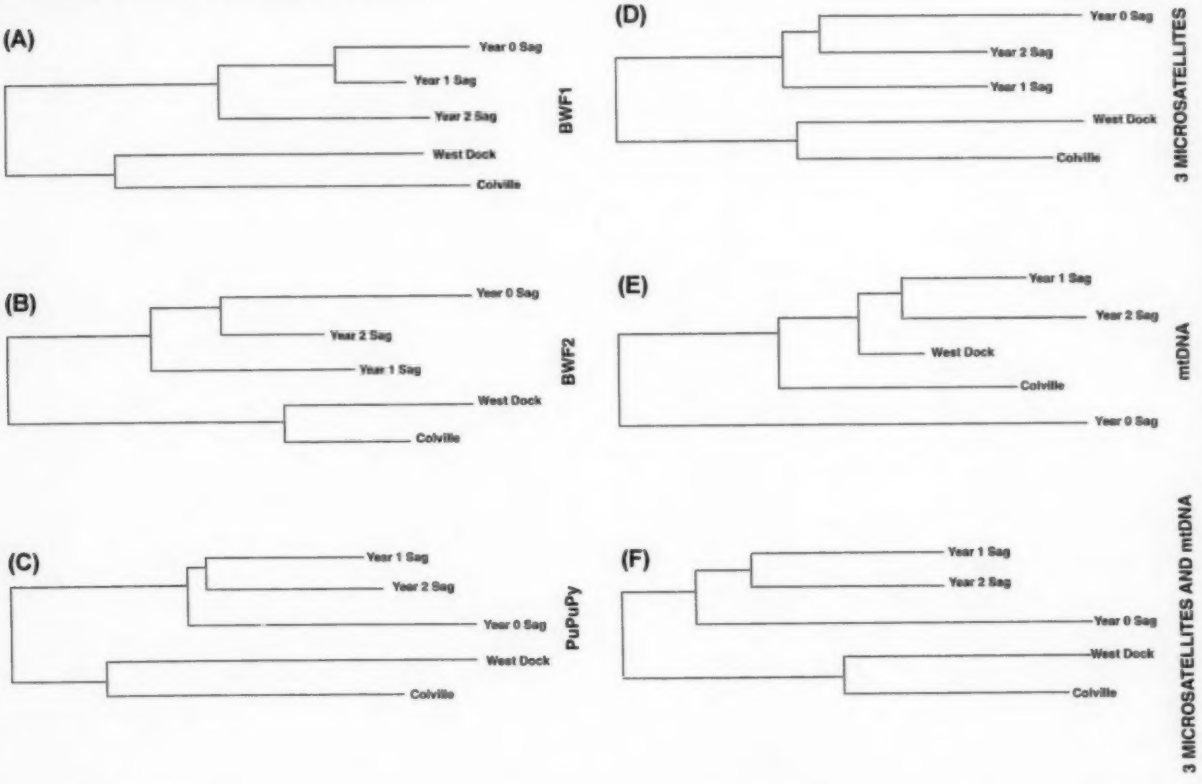
Fig. 2. Neighbor-joining dendrograms generated from Cavalli-Sforza and Edwards genetic distances for (A) BWF1 microsatellite locus; (B) BWF2 microsatellite locus; (C) PuPuPy microsatellite locus; (D) three microsatellite loci (BWF1, BWF2, and PuPuPy); (E) mtDNA ; and (F) three microsatellite loci and mtDNA combined.



We observed a lower level of differentiation of mtDNA than microsatellites between the Sagavanirktok and Colville rivers. This is contrary to the expectation of more rapid differentiation of mtDNA than nuclear genes by genetic drift, because of the smaller effective gene number of haploid, maternally inherited mtDNA (Birky et al. 1983; DeSalle et al. 1987). However, if relatively few males mate with many females, the effective gene numbers may be greater for mtDNA than nuclear genes, leading to more rapid genetic drift of nuclear loci. Although sex ratios of broad whitefish in the Mackenzie River may be as low as one male per 12 females (Wynne-Edwards 1952), sex ratios in the Alaskan Beaufort Sea are generally close to 1 male per female (Percy 1975; de Graaf and Machniak 1977; Bond and Erickson 1982; Lawrence et al. 1984; Philo and Moulton 1993*a*, 1993*b*). This suggests that more rapid genetic drift of microsatellites than mtDNA is not responsible for the pattern we observed. Different rates of gene flow, mutation, or natural selection of mtDNA and microsatellites also could result in greater differentiation of microsatellites than mtDNA. These factors may differ between mitochondrial and nuclear genes (Takahata and Slatkin 1984; Avise 1992, p. 219; Karl and Avise 1992; Adkison 1995; Ballard and Kreitman 1995; Slatkin 1995), but we do not have data to assess their influence on the broad whitefish populations. It is perhaps most important to note that the nature of variation and mode of inheritance of different types of loci must be considered when assessing population differentiation (Avise 1992; Scribner et al. 1994; Slatkin 1995). Microsatellite loci have been instituted in studies of natural population genetics relatively recently, and their evolutionary dynamics may be different from other nuclear loci.

There is genetic differentiation of the broad whitefish of the Sagavanirktok and Colville rivers, which suggests these rivers contain semi-isolated populations. This supports the idea that the changes in population numbers in the Sagavanirktok population are due to ecological factors, such as availability of overwintering habitat, and not extensive immigration and emigration. Other studies have shown genetic differentiation of broad whitefish from the Mackenzie River (J. Reist, personal communication) and for lake whitefish (*Coregonus clupeaformis*) in the Yukon River and Beringia glacial refugium (Bernatchez and Dodson 1991; Bernatchez et al. 1996). These rivers have been unglaciated for least 200 000 years and probably have populations that have differentiated over this time.

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Periphyton assemblages as indicators of recovery in acidified Canadian Shield lakes

Rolf D. Vinebrooke and Mark D. Graham

Abstract: Periphyton is very responsive to abiotic changes within recovering acidified shield lakes. We conducted a survey of acidified shield lakes to assess epilithic periphyton assemblages as indicators of natural recovery. Canonical correspondence and regression analyses both showed that dissolved inorganic carbon (DIC) was the best predictor of taxonomic composition, biomass accrual, and species richness in lakes of low alkalinity. Dissolved organic carbon (DOC) was also a significant predictor of taxonomic composition, and biomass accrual (desmids, filamentous green algae, and total). Constrained indicator species analysis identified *Actinotaenium cucurbita*, *Cylindrocystis brebissonii*, *Eunotia bactriana*, *Fragilaria acidobiontica*, *Homoeothrix juliana*, *Tetmemorus laevis*, and *Zygogonium ericetorum* as indicators of high-acidity (pH <5.0), clearwater lakes with low (<0.3 mg/L) DIC concentrations. *Achnanthes minutissima*, *Cymbella microcephala*, and *Gomphonema acuminatum* were indicative of low-acidity (pH >5.6) lakes with higher DIC concentrations (>0.6 mg/L) and positive buffering capacities. Our findings suggest that periphyton in recovering acidified shield lakes are stimulated by increasing DIC and DOC concentrations. Increases in DIC availability might enhance recolonization by diatoms in clearwater lakes, while increases in DOC concentrations may favour desmids and filamentous zygnematacean taxa, especially in naturally acidic brown-water lakes.

Résumé : Le périphyton est très sensible aux changements abiotiques survenant dans les lacs de bouclier acidifiés. Nous avons réalisé un relevé des lacs de bouclier acidifiés pour évaluer les assemblages de périphyton épilithique comme indicateurs du rétablissement naturel. Des analyses de correspondance canonique et de régression ont révélé que le carbone inorganique dissous (CID) était le meilleur prédicteur de la composition taxinomique, de l'accumulation de biomasse totale et de la richesse en espèces dans les lacs de faible alcalinité. Le carbone organique dissous (COD) était également un prédicteur de la composition taxinomique et de l'accumulation de biomasse totale (desmidés, algues vertes filamenteuses et total). L'analyse des espèces indicatrices avec contraintes a identifié *Actinotaenium cucurbita*, *Cylindrocystis brebissonii*, *Eunotia bactriana*, *Fragilaria acidobiontica*, *Homoeothrix juliana*, *Tetmemorus laevis* et *Zygogonium ericetorum* comme des indicateurs de lacs à eau limpide d'acidité élevé (pH <5,0) ayant une faible concentration de CID (<0,3 mg/L). *Achnanthes minutissima*, *Cymbella microcephala* et *Gomphonema acuminatum* étaient des organismes indicateurs de lacs de faible acidité (pH >5,6) ayant des concentrations de CID plus élevées (>0,6 mg/L) et une capacité tampon positive. Nos résultats indiquent que le périphyton dans les lacs de bouclier acidifiés en voie de rétablissement est stimulé par des concentrations croissantes de CID et de COD. Une augmentation de la disponibilité du CID pourrait accroître la recolonisation par les diatomées dans les lacs à eau limpide, tandis qu'une augmentation des concentrations de COD pourrait favoriser les taxons de desmidés et de zygnématacées filamenteuses surtout dans les lacs aux eaux brunes naturellement acides.
[Traduit par la Rédaction]

Introduction

The recent chemical recovery of many acidified Canadian Shield lakes has prompted studies into their biological recovery (Griffiths and Keller 1992; Gunn and Keller 1990; Keller et al. 1992*a*, 1992*b*; Locke et al. 1994). Microbial communities are expected to be the most responsive to recovery, owing to short generation times and a lack of dispersal barriers (Schindler 1987; Yan and Welbourn 1990; Vinebrooke 1996). Studies of phytoplankton (Nicholls et al. 1992; Findlay and Kasian 1996) and fossil diatom assemblages (A.S. Dixit et al. 1992; S.S. Dixit et al. 1992) have already shown that algal assemblages can recover from cultural and experimental lake acidification.

The responsiveness of periphyton to anthropogenic acidification and neutralization suggests that it is a valuable bioindicator (Stokes and Howell 1988). Photosynthesis and biomass accrual are suppressed by lake acidification owing to reduced dissolved inorganic carbon (DIC) concentrations (Turner et al. 1987, 1991; Fairchild and Sherman 1992), which favours loosely attached zygnematacean algae and reduces taxonomic richness (Müller 1980; see review in Stokes 1986). Furthermore, the inhibitory effects of ultraviolet radiation on periphyton (Bothwell et al. 1993, 1994; Vinebrooke and Leavitt 1996) may be particularly pronounced in acidified lakes owing to resulting declines in dissolved organic carbon (DOC) content (Schindler et al. 1996; Yan et al. 1996).

Changes in taxonomic composition may be among the best early indicators of biotic responses to lake acidification (Schindler 1987). For example, lake acidification causes

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Table 1. The pH histories of the 15 lakes surveyed in Killarney Park Provincial Park, Ontario.

Lake	1971–1973	1976–1980	1981	1988	1990	1992	ΔpH 1971–1992
A.Y. Jackson (AY)	5.0	5.4	5.4	5.6	6.1	5.6	0.6
Carlyle (CA)	5.0	5.3	5.7		6.6	5.1	0.1
Clearsilver (CS)	4.3				4.8	4.4	0.1
Freeland (FR)	4.2				6.0	4.8	0.6
Heaven (HE)						3.9	
Kakakise (KA)	5.6	6.1	7.5		6.5	6.1	0.5
Little Sheguiandah (LS)	5.0				6.1	6.3	1.3
Log Boom (LB)	5.3				5.3	5.1	–0.2
Norway (NO)	4.7	4.4			5.1	4.5	–0.2
Partridge (PA)	5.2				5.1	5.0	–0.2
Ruth-Roy (RR)	4.5	4.6	4.6	4.7	5.1	4.2	–0.3
Sandy (SA)	4.2					4.7	0.5
Silver (SI)			4.1	4.2		4.1	
Terry (TE)	4.2	4.6	4.6		5.9	4.7	0.5
Wagon Road (WR)						5.4	

Note: Data were compiled from Sprules (1975), Bleiwas et al. (1984), Dixit et al. (1989), Locke et al. (1994), and Vinebrooke (1996). Lake codes used in the ordinations are given in parentheses.

unique taxonomic changes in periphyton below pH 5.6 (Turner et al. 1995*b*). In comparison, the presence of a functional algal group, such as a filamentous green algal mat, may be inadequate as an indicator of lake acidification as this group is not restricted to only culturally acidified lakes (Howell et al. 1990) but also occurs in dystrophic (Yung et al. 1986) and certain circumneutral lakes (Hoshaw and McCourt 1988). Unfortunately, wide variation in methodology and a lack of taxonomic detail have prevented the compilation of a comprehensive list of periphyton indicator species for lakes that are sensitive to anthropogenic acidification (Stokes and Howell 1988).

We conducted a survey of periphyton across an acidification gradient that consisted of 15 culturally acidified lakes in central Ontario. Many of these lakes have undergone substantial chemical recovery (~1 pH unit) from industrial acidification since the late 19th century (A.S. Dixit et al. 1992; S.S. Dixit et al. 1992). However, a comparison of recent reports of water chemistry suggest that recovery has been highly variable across these lakes (Table 1). The objectives of the data analyses were threefold. We determined the abiotic factors that best predicted patterns in biomass accrual by major periphyton groups. Also, we determined the abiotic factors that best defined the environmental gradients along which periphyton assemblages were distributed. Finally, we classified periphyton communities and identified indicator species and lakes that represented different states of recovery from acidification.

Methods

Study lakes

The survey lakes are located in Killarney Provincial Park (46°3′N, 81°21′W), a wilderness park approximately 60 km southwest of Sudbury, Ont., Canada. Lakes are situated among predominantly quartzite and granitic ridges of the LaCloche Mountains (Debicki 1982). Lake selection was restricted to lakes of low alkalinity (Table 2), as identified from earlier surveys by Sprules (1975) and Bleiwas et al. (1984).

Data collection

Epilimnetic water samples were collected from the main outflow of each lake in mid-May and mid-September 1992. We sampled at lake outflows to obtain integrated water chemistries for the littoral area of each lake. Further details of the protocol used in water sampling and chemical analysis are given elsewhere (Vinebrooke 1996). Briefly, acid-neutralizing capacity (ANC), pH, and conductivity of unfiltered water samples were measured in the field (Wetzel and Likens 1991), while filtered (Whatman GF/F) samples were stored in ice for metal and nutrient analyses in the laboratory (Ontario Ministry of the Environment 1981). DIC and DOC concentrations were measured by total carbon infrared gas analysis (Kaplan 1992) on unfiltered and filtered (0.45 μm) water samples, respectively.

Ten unpolished quartzite tiles (10 × 10 cm) were individually deployed at near-vertical inclinations along submerged (~0.5 m) ledges in the granitic and quartzite bedrock of sheltered bays within each lake during early May 1992. The use of quartzite tiles was intended to simulate the predominant naturally occurring substrate upon which epilithon develops within these lakes. The placement of the tiles was also intended to establish a consistent habitat for periphyton development, namely a nonerosional environment with low rates of deposition and nutrient recycling (see Turner et al. 1994). Tiles were retrieved and sampled in mid-September to allow for maximal successional development of the periphyton. Monthly sampling of tiles from three of the survey lakes (A.Y. Jackson, Little Sheguiandah, Ruth-Roy) showed periphytic biomass accrual and taxonomic diversity had peaked by early September (M.D. Graham and R.D. Vinebrooke, unpublished data). A plastic container was placed directly over each tile to minimize the loss of loosely attached algae. Periphyton was easily removed with a glass scraper and hard-bristle toothbrush, and preserved with Lugol solution (Aloi 1990).

Periphytic biomass and taxonomic composition were quantified at 250–1000× using Utermöhl chambers. Most taxa were enumerated at 400×. Taxonomic richness, large colonial taxa, and filamentous taxa were enumerated separately at 250× to improve accuracy (Stevenson and Lowe 1986). Chloroplast integrity was used to determine cell viability. A minimum of 300 viable algal units were enumerated per sample. Algal biovolumes were estimated from density determinations using geometric formulae for the appropiate shapes (Wetzel and Likens 1991). Diatom biovolumes were determined using planimetric measurement of valve surface areas multiplied by girdle widths.

Major taxonomic references were Croasdale et al. (1983), Patrick and Reimer (1966, 1975), Prescott (1982), and Prescott et al. (1972,

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Table 2. Limnological conditions of the 15 survey lakes sampled in mid-May and mid-September during 1992 in Killarney Provincial Park, Ontario, Canada.

Lake	Latitude (°N)	Longitude (°W)	Al (μg/L)	ANC (μequiv./L)	Conductivity (μmhos/cm)	Ca (mg/L)	DIC (mg/L)	DOC (mg/L)	Mg (mg/L)	P (μg/L)	Secchi depth (m)	Z_{max} (m)
A.Y. Jackson (AY)	46°2′	81°24′	108	2	22.0	1.97	0.27	3.78	1.28	9.5	7.5	9.8
Carlyle (CA)	46°4′	81°13′	125	1	25.5	2.16	0.52	5.16	1.29	7.7	5.5	14.6
Clearsilver (CS)	46°7′	81°15′	314	–43	24.5	0.82	0.13	1.74	0.71	7.3	12.5	13.7
Freeland (FR)	46°2′	81°22′	123	–24	25.5	2.01	0.25	2.99	1.19	7.2	2.0	2.0
Heaven (HE)	46°4′	81°18′	299	–99	23.5	0.34	0.18	8.64	0.42	8.2	4.1	4.8
Kakakise (KA)	46°4′	81°20′	74	26	28.0	2.05	0.64	2.67	1.44	8.2	9.5	30.5
Little Sheguiandah (LS)	46°2′	81°23′	77	75	26.5	1.86	1.04	5.41	1.74	8.2	2.5	2.7
Log Boom (LB)	46°7′	81°14′	114	–9	27.5	2.35	0.32	3.44	1.32	7.8	3.2	6.0
Norway (NO)	46°5′	81°19′	207	–39	23.5	1.39	0.09	0.95	1.05	7.3	15.5	33.6
Partridge (PA)	46°5′	81°18′	116	–17	24.5	2.68	0.32	2.74	1.34	8.7	12.5	16.8
Ruth-Roy (RR)	46°5′	81°15′	332	–52	25.0	0.71	0.07	0.93	0.65	7.9	15.5	18.0
Sandy (SA)	46°5′	81°16′	178	–19	22.5	1.42	0.08	1.08	0.65	8.2	14.2	42.7
Silver	46°6′	81°16′	292	–48	21.0	0.56	0.14	1.09	0.51	6.9	12.5	15.0
Terry (TE)	46°4′	81°17′	207	–12	23.0	1.44	0.29	6.55	1.03	7.6	2.1	8.0
Wagon Road (WR)	46°1′	81°21′	106	31	19.0	1.49	0.99	10.16	1.40	8.3	1.1	2.1

Note: Unfiltered water samples were used to measure acid-neutralizing capacity (ANC), conductivity, and dissolved inorganic carbon (DIC). Al, Total dissolved (<0.45 μm) aluminum; DOC, dissolved organic carbon.

1975, 1977, 1981). The identification of diatoms was aided by preparing reference slides of cleared frustules mounted in Hyrax™. Kadlubowska (1984) and Wei et al. (1989) were used to identify filamentous zygnematacean algae and to differentiate *Zygogonium ericetorum* from *Z. tunetanum*. *Zygogonium ericetorum* consisted of small (12–15 × 15–20 μm), thick-walled vegetative cells that were separated by swollen H-shaped crosswalls and contained purplish cytoplasm and heavily distorted chloroplasts. Asexual reproduction by thick-walled parthenospores (15–20 μm diameter) was also often observed in samples of *Z. ericetorum*. Culturing of *Z. ericetorum* confirmed that the distorted appearance of its cell contents were not attributable to either changes in acidity or aluminum concentrations and that its purplish pigmentation diminished under low-light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) conditions (Vinebrooke 1994). In comparison, maintained samples of *Z. tunetanum* from the Killarney lakes had consistently larger cell dimensions (15–40 × 20–120 μm), nonpigmented cells that contained paired stellate chloroplasts and, occasionally, taxonomically diagnostic sexual reproductive structures.

Statistical analysis

Chemical variables and periphyton biovolumes were $\log_{10}$ transformed prior to statistical analysis to stabilize variances and approximate normality (Zar 1996; Morin and Cattaneo 1992). Pearson product–moment correlations with Bonferroni-adjusted probabilities were used to detect significant environmental covariables. Simple and stepwise backward multiple linear regression analyses (Zar 1996) were performed to detect significant predictive relationships between environmental variables and biovolume accrual by periphyton and its major taxonomic groups.

Taxa that occurred in at least three lakes were included in the classification and ordination analyses. This excluded taxa that had little influence on these analyses owing to a lack of variation in their distributional abundances (ter Braak 1987). Logarithmic transformation of average taxon-specific biovolumes was performed to downweight the few large-celled taxa, such as filamentous green algae, that can otherwise dominate classification and ordination analyses (van Tongeren 1987).

Ordinations were performed using the computer program CANOCO version 3.12 (ter Braak 1991). Correspondence analysis (CA) was used to capture the maximum variance within the taxonomic biovolume data set. Canonical correspondence analysis (CCA; ter Braak 1986) was performed to determine the combinations of environmental variables that best explained taxonomic patterns in periphyton assemblages. Environmental data were screened for insignificant and redundant variables using the protocol of Hall and Smol (1992), which prevented the distorting "arch effect" that can occur if superfluous variables are used in CCA. Briefly, a preliminary CCA using all environmental variables was run with forward selection to rank the variables in terms of the proportion of taxonomic variance explained by each. Then a series of partially constrained CCAs was performed that tested each top-ranked environmental variable for an independent and significant influence on the ordination. In each partial CCA, a significant covariable of a higher ranked environmental variable was entered as the sole covariable and its independence determined using Monte Carlo permutation testing for the significance of the first canonical axis. This step removed environmental covariables that were redundant. Independent environmental variables were also tested for significance using partial CCA. Following the elimination of insignificant and redundant environmental variables, a CCA was performed and inspected for variables that had high variance inflation factors (>20). Such variables were eliminated from the final CCA as they have unstable canonical coefficients that did not merit interpretation (ter Braak 1991). The significance of the final CCA axes were determined by Monte Carlo permutation testing of the eigenvalues (ter Braak 1991). Ordinations using $\log_{10}$-transformed taxon-specific biovolumes and presence–absence taxonomic data were compared to assess both the robustness of our ordinations and whether weighting by biovolume better explained periphyton–environment relationships.

Classification of periphyton assemblages and the identification of indicator taxa were performed using the divisive clustering technique contained within the computer program COINSPAN version 0.5 (Carleton et al. 1996). Constrained indicator species analysis (COINSPAN) is a revision of two-way indicator species analysis (TWINSPAN; Hill 1979), that supports direct analysis of environmental gradients. COINSPAN performs a series of divisions of the CCA axes to classify species and lakes into groups according to their ecological preferences and limnological conditions, respectively. Species abundances are first translated into the presence of one or more pseudospecies as defined by a series of increasing abundance limits, or "cut levels" (see van Tongeren 1987). Each division is then constructed based on differences between the weighted averages of the frequencies of the pseudospecies on each side of the dichotomy. Species that show strong preferences for one side of each division are

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Fig. 1. Relative algal composition of periphyton in 15 shield lakes along an acidity gradient located in Killarney Provincial Park, Ontario. Lake are arranged in order of increasing DIC concentration. Lake codes are given in Table 1.

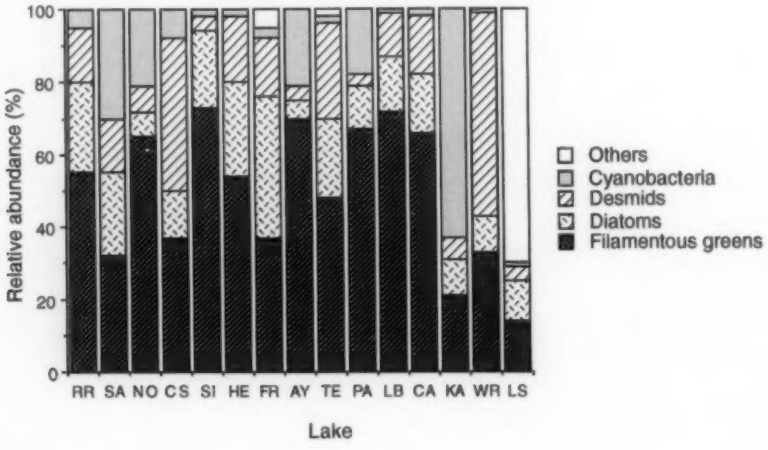

Table 3. Summary of significant relationships between DIC or DOC and biomass accrual by major periphyton groups and taxonomic richness.

Equation	r^2	$F_{1,13}$	P
Total = $3.5DIC^{0.61}$	0.48	10.6	<0.01
Total = $2.9DOC^{0.58}$	0.38	7.3	<0.05
Filamentous greens = $2.6DOC^{0.51}$	0.27	5.0	<0.05
Desmids = $1.8DOC^{0.83}$	0.33	6.2	<0.05
Taxonomic richness = $1.7DIC^{0.24}$	0.47	10.5	<0.001

Note: Simple regression analyses were performed using $\log_{10}$-transformed environmental and periphyton data.

identified as indicators. The set of indicator species can be used in subsequent surveys of the study area to classify lakes and thereby assess their recovery status.

Results

Periphyton biomass – environment relationships

Periphyton consisted primarily of filamentous green algae in all but the least acidic lakes (Fig. 1). For instance, the green colonial *Coleochaete scutata* was the dominant alga in Little Sheguiandah (pH ~6.3), whereas cyanobacteria, consisting mainly of *Microcystis*, were the most abundant periphytic algae in Kakakise Lake (pH ~6.1). Desmids were dominant in the periphyton of the brown-water Wagon Road Lake (pH ~5.4).

Regression analyses showed that DIC and DOC were the only significant predictors of periphyton abundance or taxonomic richness (Table 3). Periphyton biomass accrual was positively correlated with DIC (Fig. 2*a*) and DOC (Fig. 2*b*), while desmid and filamentous green biovolume accruals were significantly related to only DOC (Figs. 2*c* and 2*d*). Taxonomic richness was also directly correlated with DIC (Fig. 3). Multiple regression analysis showed that other abiotic variables did not strengthen these predictive relationships between DIC, DOC, and periphyton.

Periphyton assemblage – environment relationships

Ordinations were conducted using 72 taxa that each occurred in three or more lakes and collectively represented ~98% of the cumulative periphyton biovolume sampled across the 15 survey lakes (Table 4). Fifty-seven taxa did not meet our taxonomic selection criteria, occurring too infrequently to affect the results of these multivariate analyses.

CA of the periphyton assemblages summarized 56% of the total variance with the first four axes (Table 5). Use of pooled presence–absence data in place of log10-transformed abundance data resulted in only a small difference in the amount of explained total variance. In both cases, the length of the gradients represented by the first two CA axes exceeded 3 SD units, thereby justifying our choice of unimodal response models (CA, CCA) over linear models (principal component analysis, redundancy analysis) (ter Braak and Prentice 1988).

Data screening for redundant and insignificant environmental variables eliminated certain variables that did not influence the CCA. A preliminary CCA with forward selection ranked the variables in order of decreasing importance as DIC, DOC, Secchi depth, pH, ANC, Al, Mg, Ca, P, and conductivity. Partially constrained CCAs were performed using the top-ranked variables and their significant covariables to detect redundancies. Pairings of significantly correlated variables that were tested included DIC and pH, DOC and Secchi depth, Al and Mg, and Al and Ca (Table 6). Lake pH, Ca, and Mg were eliminated as they did not exert significantly independent influences as covariables of the higher ranked DIC and Al variables. Similarly, Secchi depth was eliminated owing to its redundancy with DOC. The remaining independent variables that did not exert significant influences on the ordination were conductivity ($P = 0.39$) and P ($P = 0.42$).

CCA using $\log_{10}$-transformed algal biovolume data generated a first axis that was significant ($P < 0.01$) and represented an acidity gradient (Fig. 4*a*). The acidity gradient was best defined by DIC ($r^2 = 0.76$), which explained 47% of the variance within the species data that was extracted by the first four CCA axes (Table 5). In comparison, Al ($r^2 = 0.64$) and

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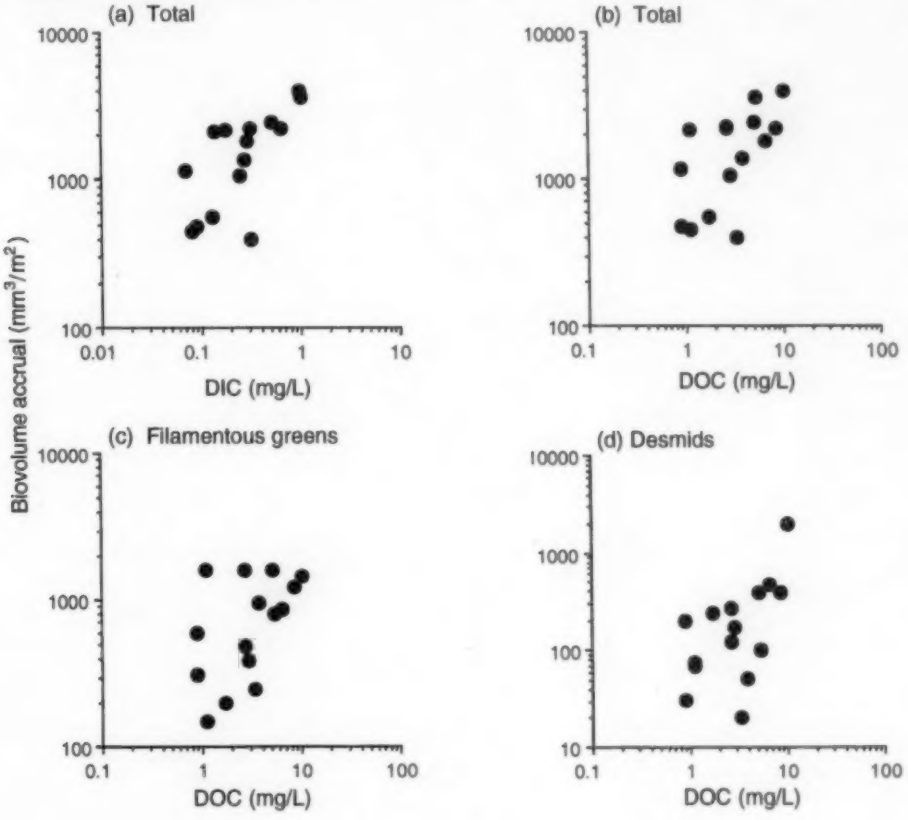
Fig. 2. Significant ($P < 0.05$) periphyton biomass – environment relationships: (*a*) total biovolume and DIC, (*b*) total biovolume and DOC, (*c*) filamentous green biovolume and DOC, and (*d*) desmid biovolume and DOC. Algal data represent mean ($n = 10$) biovolume accruals. See Table 3 for regression equations and significance levels.



DOC ($r^2 = 0.59$) were also related to axis 1, accounting for 17 and 24% of the explained variance within the species data, respectively. ANC was related to CCA axis 1 ($r^2 = 0.23$) and explained 12% of the variance. The first axis contrasted acidobiontic species, such as *Fragilaria acidobiontica* (No. 55) and *Z. ericetorum* (No. 19) on the far right, from those that preferred lakes of lower acidity located on the left side of the plot. Clearwater lakes of high acidity were strongly associated with the first CCA axis, with progressively less acidic lakes diverging from CCA axis 1 along CCA axis 2.

The second CCA axis was significant ($P < 0.05$) and best approximated a DOC ($r^2 = 0.37$) gradient (Fig. 4*a*). Many desmids, such as *Bambusina brebissonii* (No. 23), *Desmidium* (No. 27), and *Phymatodocis* (No. 34), were positively associated with the DOC vector. This axis contrasted brown-water lakes of relative high acidity from less acidic, clearwater lakes.

Use of pooled presence–absence taxonomic data generated CCA results that were comparable with those obtained using $\log_{10}$-transformed algal biolume data (Fig. 4, Table 5). CCA of presence–absence data generated a significant ($P < 0.01$) CCA axis 1 that was best explained by DIC ($r^2 = 0.79$). DIC captured 48% of the variance extracted by the first four CCA axes. DOC ($r^2 = 0.39$) best defined the second CCA axis, and accounted for 18% of the explained species data variance. Both ordinations resembled approximate mirror images of each other as lake scores from the two biplots were highly correlated along CCA axis 1 ($r^2 = 0.92$) and axis 2 ($r^2 = 0.61$).

Classification of periphyton and lakes

COINSPAN classified periphyton assemblages and lakes into four groups when $\log_{10}$-transformed taxonomic data were used (Fig. 5*a*; Table 7). DIC was the primary discriminator of periphyton assemblages and lakes, distinguishing eight relatively DIC-poor (<0.25 mg/L) lakes from a group of seven lakes that had a higher average DIC concentration of 0.58 mg/L. *Actinotaenium cucurbita* (No. 21), *F. acidobiontica* (No. 55), *Homoeothrix juliana* (No. 5), *Microspora pachyderma* (No. 11), and *Zygogonium ericetorum* (No. 19) were indicators of these low-DIC lake conditions. The group of DIC-poor lakes was subsequently divided on the basis of DOC concentration, creating a group of six relatively clearwater (DOC <3 mg/L) lakes (group 1) and a subgroup pairing of brown-water lakes (group 2). *Homoeothrix juliana* and *Z. ericetorum* were further identified as indicators of the clearwater, DIC-poor lakes (group 1), while no specific indicators existed for the brown-water, DIC-poor lakes (group 2).

Several desmids and zygnematacean species were indicators of lakes with DIC concentrations exceeding 0.3 mg/L

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Table 4. List of 72 epilithic periphyton taxa used in ordination and classification analyses.

No.	Taxon
1.	*Anabaena* spp. (C)
2.	*Aphanocapsa* spp. (C)
3.	*Chroococcus pallidus* (C)
4.	*Hapalosiphon* spp. (C)
5.	*Homoeothrix juliana* (C)
6.	*Merismopedia glauca* (C)
7.	*Phormidium tenue* (C)
8.	*Oscillatoria* spp. (C)
9.	*Rhaboderma lineare* (C)
10.	*Bulbochaete* spp. (FG
11.	*Microspora pachyderma* (FG)
12.	*Oedogonium* spp. (FG)
13.	*Coelastrum cambricum* (G)
14.	*Scenedesmus quadricauda* (G)
15.	*Pediastrum* spp. (G)
16.	*Klebsormidium subtilissima* (FG)
17.	*Mougeotia* spp. (FG)
18.	*Spirogyra* spp. (FG)
19.	*Zygogonium ericetorum* (FG)
20.	*Zygogonium tunetanum* (FG)
21.	*Actinotaenium cucurbita* (DE)
22.	*Arthodesmus extensus* (DE)
23.	*Bambusina brebissonii* (DE)
24.	*Closterium* spp. (DE)
25.	*Cosmarium* spp. (DE)
26.	*Cylindrocystis brebissonii* (DE)
27.	*Desmidium* spp. (DE)
28.	*Docidium* spp. (DE)
29.	*Euastrum* spp. (DE)
30.	*Gonatozygon* spp. (DE)
31.	*Hyalotheca* spp. (DE)
32.	*Netrium digitus* (DE)
33.	*Penium cylindrus* (DE)
34.	*Phymatodocis* spp. (DE)
35.	*Pleurotaenium* spp. (DE)
36.	*Spondylosium planum* (DE)
37.	*Staurastrum* spp. (DE)
38.	*Tetmemorus laevis* (DE)
39.	*Xanthidium* spp. (DE)
40.	*Achnanthes marginulata* (DI)
41.	*Achnanthes minutissima* (DI)
42.	*Actinella punctata* (DI)
43.	*Anomoeoneis serians* (DI)
44.	*A. serians* var. *brachysira* (DI)
45.	*Cymbella microcephala* (DI)
46.	*Cymbella lunata* (DI)
47.	*Cymbella minuta* (DI)
48.	*Eunotia bactriana* (DI)
49.	*Eunotia bidentula* (DI)
50.	*Eunotia* spp. (DI)
51.	*Eunotia pectinalis* (DI)
52.	*E. pectinalis* var. *minor* (DI)
53.	*E. pectinalis* var. *ventricosa* (DI)
54.	*Eunotia serra* (DI)
55.	*Fragilaria acidobiontica* (DI)
56.	*Frustulia rhomboides* (DI)
57.	*F. rhomboides* var. *crassinerva* (DI)
58.	*F. rhomboides* var. *saxonica* (DI)
59.	*Gomphonema acuminatum* (DI)
60.	*Gomphonema gracile* (DI)
61.	*Navicula subtilissima* (DI)
62.	*Neidium affine* (DI)
63.	*Neidium iridis* (DI)
64.	*N. iridis* var. *amphigomphus* (DI)
65.	*Pinnularia* spp. (DI)
66.	*Semiorbis hemicyclus* (DI)
67.	*Stauroneis* spp. (DI)
68.	*Stenopterobia intermedia* (DI)
69.	*Tabellaria binalis* (DI)
70.	*Tabellaria fenestrata* (DI)
71.	*Tabellaria flocculosa* (DI)
72.	*Audouinella* (R)

Note: Taxon identification numbers correspond to those used in the CCA plots. C, cyanobacterium; DE, desmid; DI, diatom; FG, filamentous green alga; G, nonfilamentous green alga; R, red alga.

Table 5. Cumulative percentage variance extracted by CA and CCA axes from the species data.

Ordination	Axis I	Axis II	Axis III	Axis IV
CA (log)	27.3	39.3	48.7	55.6
CA (+/–)	26.7	38.1	48.6	57.1
CCA (log)	24.8	34.2	41.0	47.0
CCA (+/–)	24.8	33.3	40.0	45.7

Note: CA and CCA were performed using either $\log_{10}$-transformed (log) algal biovolumes, or presence–absence (+/–) species data, along with $\log_{10}$-transformed environmental data in the case of CCA.

Fig. 3. Taxonomic richness of periphyton as a function of DIC concentration. See Table 3 for regression equation and significance level.

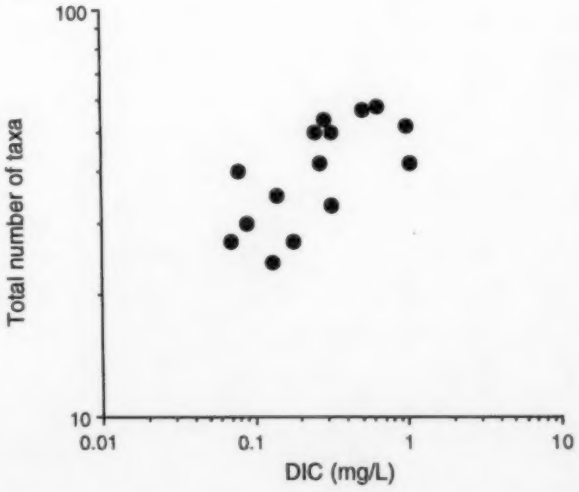

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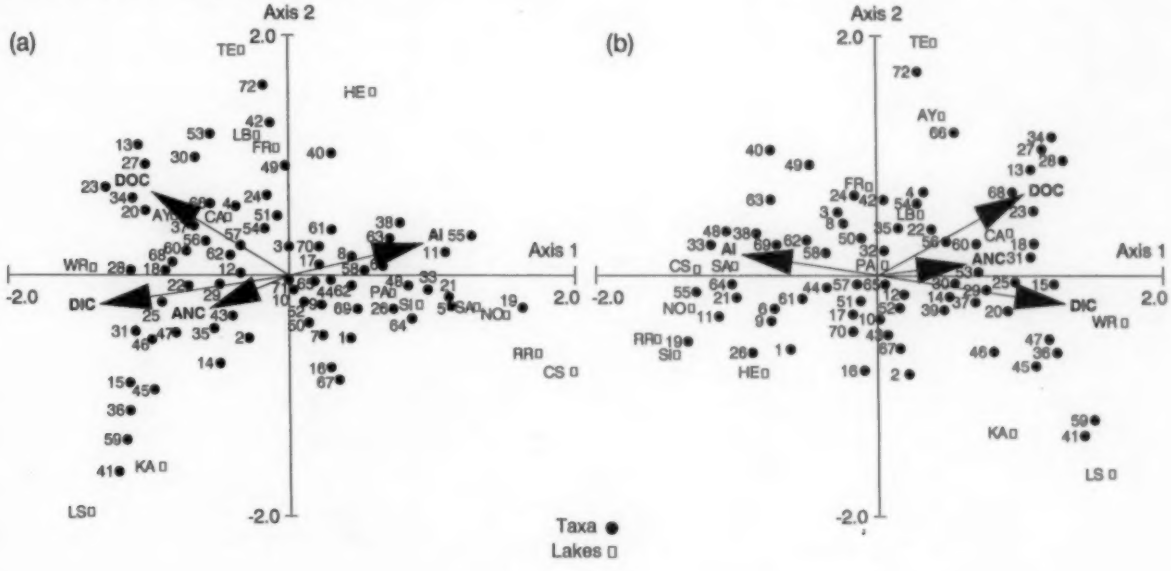
Table 6. Pearson product–moment correlation matrix for environmental variables of the 15 Killarney survey lakes.

Variable	Al	ANC	Conductivity	Ca	DIC	DOC	Mg	P	pH
Secchi depth	0.40	–0.01	0.01	–0.24	–0.58	–0.85*	–0.35	–0.03	–0.33
pH	–0.94*	0.64	0.29	0.72	0.81*	0.34	0.89*	0.49	
P	–0.47	0.03	–0.07	0.34	0.31	0.34	0.40		
Mg	–0.93*	0.74	0.27	0.91*	0.72	0.29			
DOC	–0.38	–0.18	–0.13	0.15	0.66				
DIC	–0.76	0.41	0.06	0.45					
Ca	–0.85*	0.69	0.37						
Conductivity	–0.30	0.07							
ANC	–0.58								

*$P < 0.05$ (Bonferroni-adjusted probability).

Fig. 4. Association of periphyton taxa, lakes, and water chemistry based on CCA using (*a*) $\log_{10}$-transformed taxa biovolumes and (*b*) taxonomic presence–absence data, along with $\log_{10}$-transformed water chemistry data. Lake codes are given in Table 1 and taxonomic identification numbers are given in Table 4.



(Fig. 5*a*, Table 7). However, when this group of lakes was further subdivided on the basis of ANC, no specific indicators were identified for lakes that had intermediate buffering capacities (group 3). In contrast, certain diatoms (*Achnanthes minutissima*, No. 41; *Cymbella microcephala*, No. 45; *Gomphonema acuminatum*, No. 59) were identified as indicators of lakes that had the highest DIC concentrations and positive buffering capacities (group 4).

COINSPAN identified a similar set of four lakes groups when presence–absence taxonomic data were used (Fig. 5*b*). However, the intermediate lake groups (groups 2 and 3) consisted of different member lakes when compared with those generated using $\log_{10}$-transformed data (Table 7). Log Boom Lake and Terry Lake were reclassified as DIC-poor, brown-water lakes (group 2), while A.Y. Jackson Lake and Carlyle Lake were grouped as lakes with intermediate DIC concentrations and negligible buffering capacity (group 3). As a result of the restructuring of lake groups, some different indicator species were identified (Fig. 5). *Cylindrocystis brebissonii* (No. 26), *Tetmemorus laevis* (No. 38), and *Eunotia bactriana* (No. 48) appeared as indicators of DIC-poor lakes. *Spirogyra* (No. 18) and *Audouinella* (No. 72) were identified as indicators of DIC-poor, brown-water conditions (group 2). *Spondylosium planum* (No. 36) replaced *Bambusina brebissonii* (No. 23) as an indicator of DIC concentrations that averaged 0.7 mg/L.

Discussion

Periphyton biomass in recovering acidified lakes

Our survey results showed that periphyton biomass was best related to DIC concentrations in recovering acidified lakes. This finding supports experimental evidence showing that periphyton biomass accrual in acidified lakes of low alkalinity are resource limited (Vinebrooke 1996) owing to low DIC concentrations (Fairchild and Sherman 1992). In recovering acidified lakes, increasing DIC availability might therefore offset the proliferation of loosely attached clouds of metaphyton (Howell et al. 1990; Jackson et al. 1990) as periphyton may be

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Fig. 5. Classification of lake groups and identification of periphyton indicator taxa and key discriminating environmental variables based on a COINSPAN of (*a*) $\log_{10}$-transformed taxa biovolumes and (*b*) taxonomic presence–absence data, along with $\log_{10}$-transformed water chemistry data. Lake codes are given in Table 1. For water chemistry characteristics and member lakes of each group, see Table 7.

less likely to lift off to a planktonic environment in an effort to reduce gas diffusion problems associated with the benthic boundary layer (Turner et al. 1994). Conversely, Turner et al. (1995*a*, 1995*b*) report that during lake acidification primary production shifts from the periphyton to the metaphyton, thereby helping offset oligotrophication in the littoral zone.

Periphyton abundance was also positively related to DOC in the acidified Killarney lakes. Desmids and filamentous green algae appeared to benefit from DOC, which contributed to enhanced periphyton abundance in the surveyed humic lakes. Interestingly, periphyton in naturally acidic bogs also consists primarily of filamentous zygnematacean algae and desmids (Yung et al. 1986). DOC may enhance the development of periphyton under acidic conditions by ameliorating some of the effects of acidification not associated with reduced DIC availability. For instance, DOC can offset metal toxicity and fluctuations in acidity (Kullberg et al. 1993) that have both been shown to inhibit primary productivity (Nalewajko and Paul 1985). Furthermore, DOC attenuates ultraviolet radiation that can suppress periphyton in shallow lentic habitats (Vinebrooke and Leavitt 1996). Because DOC content is reduced during anthropogenic lake acidification (Schindler et al. 1996), recovery by periphyton in acidified lakes may depend upon the reestablishment of both preacidification DIC and DOC concentrations.

Our survey did not encompass a sufficiently large phosphorus gradient to assess the importance of phosphorus to periphyton. Similarly, Charles (1985) had attributed the lack of a relationship between benthic diatoms and phosphorus in a survey of Adirondack lakes to the absence of an appreciable phosphorus gradient. Nevertheless, the development of periphyton is only enhanced by phosphorus amendments in circumneutral

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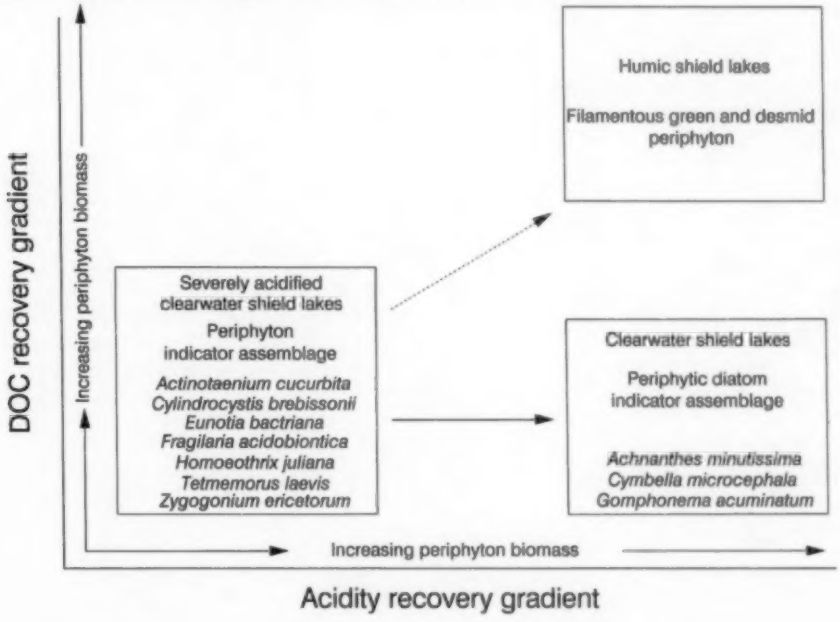
Table 7. Summary of the environmental conditions of the four groups of recovering acidified lakes identified with COINSPAN using log_{10}-transformed biovolume (log) or presence–absence (+/–) taxonomic data.

Group	Lakes	DIC (mg/L)	DOC (mg/L)	pH	Al (µg/L)	ANC (µequiv./L)
1 (log)	CS, NO, PA, RR, SA, SI	0.14	1.4	4.5	240	–36
1 (+/–)	CS, NO, PA, RR, SA, SI	0.14	1.4	4.5	240	–36
2 (log)	FR, HE	0.22	5.8	4.4	211	–62
2 (+/–)	FR, HE, LB, TE	0.26	5.4	4.6	186	–36
3 (log)	CA, LB, TE	0.38	5.1	5.0	149	–7
3 (+/–)	AY, CA	0.40	4.5	5.4	117	2
4 (log)	AY, LS, KA, WR	0.74	5.5	5.8	91	34
4 (+/–)	KA, LS, WR	0.89	6.1	5.9	86	44

Note: Water chemistry for each group was averaged from chemical analysis of mid-May and mid-September samples taken in 1992. Lake codes are given in Table 1.

Fig. 6. Potential recovery trajectories of periphyton in culturally acidified Canadian Shield lakes. Recovery for most lakes may primarily involve renewing DIC levels (along *x* axis), resulting in increased periphyton biomass and dominance by diatoms that are indicative of pristine clearwater shield lakes. Certain lakes might also recover preacidification DOC concentrations (along *y* axis) that will enhance the development of periphyton, especially desmids and filamentous greens that are indicative of humic shield lakes.



lakes in which bicarbonate concentrations offset carbon limitation (Fairchild and Sherman 1992, 1993). However, phosphorus availability does limit phytoplankton productivity in lakes of low alkalinity, while not affecting epilithic productivity (Turner et al. 1994). Periphyton in acidified lakes may avoid phosphorus limitation because of high benthic phosphatase activity or luxury consumption of phosphorus during nutrient pulses from sediments or allochthonous sources (see Fairchild and Sherman 1992; Turner et al. 1994).

Periphyton assemblages in recovering acidified lakes

Compensatory species replacements may have moderated against larger changes in biomass accrual by major algal groups along the acidity and DOC gradients. For instance, there were no significant relationships between diatom and cyanobacterial biomass and environmental variables, but each group was represented by a series of overlapping species distributions that span the major DIC and DOC gradients. Instead, significant relationships between shifts in taxonomic composition and select environmental gradients supported the view that populations, rather than major algal taxonomic groups, are the most sensitive indicators of ecosystem response to anthropogenic stresses (Schindler 1987; Cattaneo 1992; Kinross et al. 1993).

The taxonomic composition and richness of periphyton in the Killarney lakes was also best explained by DIC concentration. Although it is difficult to separate the effects of the different environmental variables related to acidity, our multivariate analyses suggested that DIC was a significant and independent variable that did explain the most variance within our taxonomic data set. In comparison, Fairchild and Sherman (1993) using CCA showed that the taxonomic composition of

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lentic periphyton was best explained by pH and alkalinity. However, unlike our study their survey covered a wider gradient of alkalinity in which some lakes were well buffered. Nevertheless, Fairchild and Sherman (1992, 1993) found that carbon enrichment enhanced the growth of many species in their acidic lakes.

Actinotaenium cucurbita, *Cylindrocystis brebissonii*, *Eunotia bactriana*, *F. acidobiontica*, *H. juliana*, *M. pachyderma*, *T. laevis*, and *Z. ericetorum* were indicators of severely acidified, DIC-poor lakes. Interestingly, many of these species have also been reported in naturally acidic bogs and humic lakes (Coesel et al. 1978; Yung et al. 1986). Only *H. juliana* and *Z. ericetorum* were specific indicators of clearwater and high-acidity conditions, showing that the other indicators in this group resided in both acidified clearwater and humic lakes. Perhaps these species colonized the acidifying clearwater Killarney lakes from nearby bogs and humic lakes that had already been naturally acidic prior to the advent of anthropogenic acid deposition.

The diatoms *Achnanthes minutissima*, *Cymbella microcephala*, and *Gomphonema acuminatum* were indicators of the least acidic lakes that had positive buffering capacities. These diatoms are frequently the dominant species in the periphyton of pristine alpine (Vinebrooke and Leavitt 1996), arctic (Moore 1979), and oligotrophic shield lakes (Stockner and Armstrong 1971). Thus, recolonization by these taxa may be a useful biological index of recovery in moderately acidified shield lakes.

Our results showed that certain filamentous zygnematacean taxa could be used as indicators in recovering acidified lakes. *Zygogonium ericetorum* was indicative of high acidity conditions (pH <5), while *Spirogyra* and *Z. tunetanum* were indicative of brown-water conditions of intermediate acidity. These findings concerning periphyton agree with reported shifts in the taxonomic composition of filamentous metaphyton assemblages in acidified lakes. Turner et al. (1995*a*) similarly showed that *Zygogonium* was the dominant metaphytic alga at pH <4.5, while *Spirogyra* dominated at pH >5. In their survey of 32 Ontario lakes, Wei et al. (1989) concluded that *Z. tunetanum* was the most common metaphytic alga in lakes below pH 6.8, but they did not report which species of *Zygogonium* predominated in the three Killarney lakes that they sampled, owing to the lack of sexual reproductive structures. We found that samples from the seven most acidic Killarney lakes contained *Zygogonium* that consistently had characteristics resembling those of *Z. ericetorum* as summarized by Kadlubowska (1984). In fact, the distorted appearance of its cell contents, along with its swollen H-shaped crosswalls, can cause *Z. ericetorum* to more closely resemble *Microspora* rather than *Z. tunetanum*. Nevertheless, field collections and samples of the two *Zygogonium* species maintained in the laboratory (Vinebrooke 1994) indicate that *Z. tunetanum* is likely succeeded by *Z. ericetorum* in lakes that experience severe acidification (pH <5).

Some periphytic taxa were poor potential indicators of lake recovery from acidification. These taxa were either relatively rare and sporadic, or ubiquitous, in their distributions across the sampled lake acidity gradient. For example, the filamentous zygnematacean taxon *Mougeotia* had no indicator value owing to its widespread distribution across the Killarney lakes, which also suggested that it had a broad tolerance of acidity. However, many filamentous taxa, including *Mougeotia*, *Bulbochaete*, and *Oedogonium*, could not be identified to species, owing to a lack of sexual reproductive structures. Similarly, Wei et al. (1989) reported only one case of sexual reproduction in 300 zygnematacean samples from this region. This unavoidable lack of taxonomic information hampers attempts at identification of certain potential indicator species. Furthermore, the use of vegetative cell dimensions in identifying zygnematacean species (e.g., Müller 1980; Kinross et al. 1993) is not recommended as these algae are polypoidal and, therefore, apt to change cell dimensions (Hoshaw and McCourt 1988). As a consequence, the ubiquity of some zygnematacean genera in acidified lakes may be due to the inability to discriminate between separate species that have overlapping acidity tolerances.

The use of $\log_{10}$-transformed abundance data and pooled presence–absence taxonomic data generated comparable ordinations. Kinross et al. (1993) also reported comparable results using these two data transformations in a survey of filamentous algal assemblages in acidic streams. Thus, direct environmental gradient analysis of periphyton assemblages based solely on taxonomic identifications do not appear to substantially benefit from the additional efforts spent on estimation of species biomass, which can be very time-consuming. As a caveat, transformation of taxonomic data did affect the classification of lake groups and identification of indicator species, indicating the need for consistency in data transformations when comparing these findings with those of other surveys of periphyton in acidified lakes.

Implications for recovering acidified lakes

Culturally acidified lakes in the Sudbury area are expected to recover by reestablishing aquatic communities that are indigenous to pristine shield lakes (Keller et al. 1992*a*). However, biological recovery can be highly variable in moderately and severely acidified lakes (Locke et al. 1994). Our findings suggest that recovery by periphyton will also vary, depending on relative increases in DIC and DOC concentrations within each lake (Fig. 6). The primacy of abiotic regulation of periphyton in these acidified lakes (Vinebrooke 1996) suggests that changes in water chemistry govern recovery rates and trajectories. Periphyton will likely respond primarily to increasing DIC concentrations but will also be influenced by increases in DOC content in recovering acidified lakes. As a result, recovery by periphyton assemblages can be characterized by a divergent set of recovery trajectories. Most recovering acidified shield lakes should reestablish circumneutral, clearwater conditions in which higher periphyton biomass accrual and taxonomic richness may be attributed to diatoms that are typically the dominant periphytic taxa in pristine shield lakes (see Stockner and Armstrong 1971). The resulting shift from DIC- to NP-limitation should also disfavour dominance by *Mougeotia*. This slow-growing alga has a high DIC uptake efficiency, but appears to be outcompeted by other algae for N and P following the liming of acidified lakes (see Fairchild and Sherman 1990; Jackson et al. 1990). Furthermore, naturally acidic lakes may regain DOC content lost during anthropogenic acidification (Schindler et al. 1996; Yan et al. 1996), resulting in more humic conditions that might favour the development of periphyton consisting mainly of desmids and filamentous zygnematacean algae.

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Spatial and temporal variation in the diet of double-crested cormorants (*Phalacrocorax auritus*) breeding on the lower Great Lakes in the early 1990s

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Abstract: We sampled 1573 pellets and 560 boli regurgitated at double-crested cormorant (*Phalacrocorax auritus*) colonies on the three lower Laurentian Great Lakes (Lake Ontario, Lake Huron, and Lake Erie) during the breeding season in 1992 and 1994. This constitutes the first extensive study of cormorant diet in this region. We found significant spatial and temporal heterogeneity in diet among colonies within a lake. Differences in diet among colonies were usually consistent with knowledge of changes in fish behaviour during the cormorant breeding season, differences in the surrounding bathymetry among breeding colonies, and the proximity of colonies to the preferred habitat of prey species. Our results indicate that temporal and spatial variation, as well as the technique for diet determination, are very important factors that must be considered when assessing the effects of double-crested cormorant predation on fisheries.

Résumé : Nous avons échantillonné 1573 pelotes et 560 bolus régurgités dans des colonies de cormorans à aigrettes (*Phalacrocorax auritus*) dans les trois Grands Lacs laurentiens d'aval (lacs Ontario, Huron et Érié) durant la saison de reproduction de 1992 et de 1994. Il s'agit de la première étude exhaustive du régime alimentaire du cormoran dans cette région. Nous avons constaté une hétérogénéité spatiale et temporelle importante dans le régime alimentaire entre les colonies dans un même lac. Les différences dans le régime alimentaire entre les colonies étaient habituellement en accord avec la connaissance des changements touchant le comportement des poissons durant la saison de reproduction du cormoran, avec les différences dans les caractéristiques bathymétriques environnantes parmi les colonies de nidification et avec la proximité des colonies par rapport à l'habitat de prédilection des espèces proies. Nos résultats indiquent que la variation temporelle et spatiale, ainsi que la technique utilisée pour déterminer le régime alimentaire, sont des facteurs très importants qui doivent être pris en considération au moment de l'évaluation des effets de la prédation du cormoran à aigrettes dans les pêcheries. [Traduit par la Rédaction]

Introduction

Cormorant species occur throughout the world in marine and freshwater ecosystems and are important predators of fish. Resource managers need reliable information on the impact of cormorants on fish stocks, but in many areas these data are not yet available. This is the first extensive study on the diet of double-crested cormorants (*Phalacrocorax auritus*) on the three lower Great Lakes.

Numbers of double-crested cormorants have increased greatly throughout North America in the last 20 years (Hatch 1984; Vermeer and Rankin 1984; Chapdelaine and Bédard 1995). On the Great Lakes in particular, double-crested cormorants have increased dramatically since the early 1970s, at an average annual rate of 29%, to about 38 000 occupied nests in 1991 (Weseloh et al. 1995). This population growth is largely attributed to declines in human persecution, an abundance of forage-base fish, and reduced levels of DDE and other organochlorine contaminants in cormorants, which had caused thinning and breakage of eggshells, deformities, and reproductive failure (Weseloh et al. 1983, 1995; Ludwig 1984; Craven and Lev 1987; Ludwig et al. 1995).

Double-crested cormorants feed almost entirely on fish (Hobson et al. 1989; Ludwig et al. 1989; Orta 1992; Campo et al. 1993), with the daily food intake of an adult double-crested cormorant in the wild estimated at 0.25–0.50 kg/day (Schramm et al. 1984, 1987), or up to about 25% of body mass (Dunn 1975). These birds forage mainly by pursuit diving (Orta 1992), on the Great Lakes usually in water depths up to about 10 m (Custer and Bunck 1992; P.J. Ewins, D.V. Weseloh

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and K. Karwowski, personal observation). They breed in many parts of North America (Orta 1992), and most are present on the Great Lakes from April to mid-October (Weir 1989; Weseloh and Ewins 1994). They nest in dense colonies usually on the ground on small rocky islands but also in tall deciduous trees when they are available (Weseloh et al. 1995).

The diet of double-crested cormorants, coupled with their rapid population growth, has increased concern among commercial and sport fishermen who believe that cormorants will compete with them for economically important fish (DesGranges and Reed 1981; Craven and Lev 1987; Ludwig et al. 1989). However, double-crested cormorants are not reported to consume significant amounts of fish with commercial or sport value (Lewis 1929; Roney 1979; Craven and Lev 1987; Hobson et al. 1989; Ludwig et al. 1989; Campo et al. 1993; Karwowski et al. 1994; Blackwell et al. 1995; Ross and Johnson 1995; Suter 1995; Johnson and Ross 1996). There is little evidence that breeding double-crested cormorants are significant competitors for commercially important predatory fish (Weseloh and Casselman 1992; Madenjian and Gabrey 1995). Similar conclusions have been reached in studies of cormorant diets in other parts of the world (e.g., Linn and Campbell 1992; Marquiss and Carss 1994). Despite these findings, considerable caution is needed when making inferences about cormorant diet based on studies with limited sampling across time or space. For instance, localized predation of sport fish at stock release sites can sometimes be heavy (Ross and Johnson 1995; P.J. Ewins, D.V. Weseloh, and K. Karwowski, personal observations; J.R. Lovvorn, personal communication).

Our study addresses the extent of temporal and spatial variation in the diet composition of double-crested cormorants on the Great Lakes using regurgitated material collected from Lakes Ontario, Huron, and Erie in the early 1990s. Specifically, we examine variation in diet among colonies on individual lakes, variation in diet over the course of a breeding season, and differences between the diets of adult and nestling cormorants. The regurgitated material includes both pellets of indigestible material produced by adults and boli of recently consumed food regurgitated by nestlings, usually when disturbed.

Study areas

In this study we refer to Lakes Huron, Erie, and Ontario as the lower Great Lakes. Pellets and (or) boli of double-crested cormorants were collected during the nesting season from colonies on Lake Ontario (1992), Lake Huron (1994), and Lake Erie (1994). On Lake Ontario, we studied colonies on Little Galloo (43°53′N, 76°24′W), Pigeon (44°04′N, 76°33′W), and Snake (44°11′N, 76°32′W) islands. On Lake Huron, we collected data from colonies on Manitoba Reef (45°42′N, 82°58′W), Gull Rock (46°06′N, 82°19′W), Bustard Islands (South-East Rock; 45°53′N, 80°57′W), and Birnie Rocks (45°26′N, 80°28′W). We collected only boli from East Sister Island (41°49′N, 82°51′W) on Lake Erie.

Methods

A total of 1573 pellets was collected from nest rims, ground immediately surrounding the nests, and from rock perches around the colony during the egg and nestling stage at intervals of 1–3 weeks, depending on the individual colony (Table 1). Only moist, intact, fresh-looking pellets were collected; dessicated, older pellets were avoided. While the chronology of the double-crested cormorant nesting season can be variable among years (Craven and Lev 1987), egg laying on Lake Ontario generally begins in late April to early May, and the main nestling stage occurs from early June to late July (Weseloh and Ewins 1994). The nesting season usually begins 7–10 days later on Lake Huron (Ewins et al. 1995).

Pellets of indigestible material (e.g., bones and scales) are thought to be produced by adult double-crested cormorants almost daily (Craven and Lev 1987), whereas chicks only start producing pellets around fledging time (Orta 1992). Although adult and nestling cormorants may regurgitate a bolus if they are disturbed while digesting, we only collected boli from nestlings. A total of 560 boli was collected in our study (Table 2). Collections were made on up to five separate occasions from some colonies.

At the time of collection, each pellet or bolus was sealed in a plastic bag and stored at –20°C usually within 10 h of collection. The pellets were later thawed, then soaked in water or a solution of water and dishwashing detergent (4 L water : 3 mL detergent) for about 24 h, which was thought to help separate fish bones from the gelatinous matrix. Diagnostic material was separated from the pellet with the aid of forceps and a dissecting microscope and air dried. Pellet contents were either rinsed carefully with water while separating diagnostic material, or the intact pellet was washed in a 250 µm mesh sieve before separating diagnostic material.

Both fish and non-fish items were identified in pellets and boli. Fish items were usually identified to the species level using diagnostic structures such as otoliths, preopercular bones, teeth, scales, and pelvic girdles. The minimum number of individual fish in a sample was determined for pellets by pairing otoliths or other diagnostic bones. Items considered not to have been ingested as food were only identified to basic categories (e.g., stones, vegetation, eggshell). In the case of bolus samples, largely intact portions of fish and non-fish items were available for identification. Many food items were identified by comparisons with material in reference collections, compiled by the Canadian Wildlife Service (Ontario Region), and U.S. Fish and Wildlife Service (Cortland, N.Y., Field Office). Keys and guides (Lagler 1947; Scott and Crossman 1973) were also used. Popular and scientific names of fish species identified are given in Appendix 1. The length of intact, undigested fish regurgitated by nestlings was measured from the tip of the snout to the most distal tip of the tail. Identification of prey species was completed for most Little Galloo Island samples by M.P., L. Smith, and S. Heiligman. Samples from other sites were identified by J.N. except Lake Erie (P.J.E.). We cross-checked our identification techniques with each other and also verified identifications for a sample of 50 pellets from Little Galloo Island, re-analyzed by experts at the American Museum of Natural History. No misidentifications were apparent from these analyses.

Statistical comparisons were made using the G test (Zar 1984) with a 0.05 level of significance. All data are reported as percent frequency of occurrence (the percentage of pellets or boli from a given location and date in which a specific food group occurred). Analyses were also performed for most data sets expressed as percent composition (based upon the minimum number of individuals of given prey types). Results were usually broadly similar to those obtained using frequency of occurrence of prey items. However, we do not report abundance data, since they often tend to provide less conservative estimates of differences in diet composition among sampling dates and colonies (see Duffy and Jackson 1986; Irons 1987; Ewins et al. 1994). The minimum number of pellets or boli for inclusion in statistical analyses for any colony on a given date was 18, but in many cases sample sizes were considerably larger than this (Table 1). Our preliminary work had indicated that a sample size of 15–20 pellets per site or date provided similar results for percent diet composition as larger numbers of pellets or boli.

Groupings of fish food types were usually at the species level with

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Table 1. Collection dates, sample sizes for pellets, and apparently occupied nests (given in parentheses) from double-crested cormorant colonies, 1992–1994.

		Lake Ontario			Lake Huron			
Month and date		L. Galloo (5433)	Pigeon (782)	Snake (284)	Gull (395)	Bustard (1013)	Birnie (384)	Manitoba Reef (879)
				1992				
April	22		15	6				
	26	54						
	28		15	18				
May	4	50	15	15				
	11	90						
	12		15	15				
	13	52						
	19	93						
	25	93	15	15				
June	1	109	15	15				
	8		15	6				
	11	53						
	14		2					
	16	73						
	22	70						
July	2	98						
	16	103						
				1994				
May	4						36	
	8				32			
	10						36	
	13					36		
	22							36
	25					36		
	27						35	
June	15							36
	17						28	
	20					36		
	26					36		
July	8						36	
	13							10
	14				3			
	19						28	
	27					14		
Total		938	107	90	35	158	163	82

the exception of Centrarchidae, Gasterosteidae, and *Notropis* sp., which were often difficult to identify beyond the family or genus level. Identified fish groups that occurred too infrequently to be included as separate categories were lumped into a single "other identified fish" group. Unknown fish were not included in comparisons of diet composition unless the occurrence of the remaining identified fish items was too small to meet the assumptions of a *G* test (Zar 1984). In the above situation, all the remaining fish were lumped into an "other fish items" group. Groupings of fish taxa were sometimes varied among statistical analyses to meet minimum sample size requirements of *G* tests (Zar 1984). Crayfish were included as a food item in statistical analyses of Birnie Island and Bustard Islands since they occurred in sufficiently large numbers for the *G* test.

Temporal comparisons of adult diet from pellets between the egg and nestling stage, and within these stages, were made only for Little Galloo Island, Birnie Rocks, and Bustard Islands; these colonies had greater than 18 pellets per sampling date and at least two sampling dates during both stages. Within the nestling and egg stage, successive sampling dates were separated by at least 1 week. The food categories used for statistical analyses between sampling dates were kept the same when comparing diet composition at different stages for consistency, despite the fact that larger sample sizes after pooling may have allowed more food categories to be introduced. Little Galloo, Pigeon, Snake, and Bustard islands were the only colonies used for temporal comparisons of nestling diet since they had multiple sampling dates with at least 18 bolus samples on each date.

Results

We collected 1135 and 464 double-crested cormorant pellets from islands on Lake Ontario (1992) and Lake Huron (1994), respectively. In Lake Ontario pellets we found remains of fish from 13 families, including 26 identified species. In Lake Huron pellets 12 fish families were represented, including 15 identified species (Appendix 2). In addition, boli were collected from Lake Ontario (1992; 283 boli; four fish families, five identified species), Lake Huron (1994; 209 boli; 12 fish

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Table 2. Collection dates and sample sizes for boli from double-crested cormorant colonies, 1992–1994.

Month and date	Lake Ontario			Lake Erie,	Lake Huron			
	L. Galloo	Pigeon	Snake	E. Sister	Gull	Bustard	Birnie	Manitoba Reef
				1992				
June 8	31	18						
14	21	23	9					
19		18						
21	35	22	25					
29	31	24	25					
				1994				
June 17							26	
20						47		
23				69				
26						33		
July 8							14	
13								41
14					47			
17								
19							1	
Total	118	105	59	69	47	80	41	41

families, 18 identified species), and Lake Erie (1994; 69 boli; 6 fish families, 8 identified species; Appendix 3). Remains of crayfish species were found regularly in pellets from Lakes Ontario and Huron but only rarely in boli (Appendices 2 and 3). Other non-fish items were detected in small quantities in small numbers of pellets and boli, but many of these items were thought to have adhered to the outside of a sample or to have been present within the stomachs of fish prey. Based on both pellets and boli, the diet of double-crested cormorants appeared to be heterogeneous, both spatially and temporally.

Temporal variation

There were differences in the food items occurring in pellets between the egg and nestling stage at Little Galloo Island ($G = 77.89$; df = 5; $p < 0.001$), Birnie Rocks ($G = 75.72$; df = 8; $p < 0.001$), and Bustard Islands ($G = 24.05$; df = 8; $p < 0.005$) (Fig. 1). At Little Galloo Island, the occurrence of centrarchids (Centrarchidae), white perch, and yellow perch decreased by approximately 20% from the egg to the nestling stage. At Birnie Rocks, centrarchids, rainbow smelt, and yellow perch showed large decreases (i.e., at least 20%) while slimy sculpin, trout-perch, and crayfish species increased in occurrence from the egg to the nestling stage. At Bustard Islands, white sucker, and yellow perch decreased greatly in occurrence from the egg to the nestling stage.

There were differences in diet composition, determined from pellets, among sampling periods within the egg stage at Little Galloo Island ($G = 92.23$; df = 30; $p < 0.001$), Birnie Rocks ($G = 36.18$; df = 16; $p < 0.005$), and Bustard Islands ($G = 18.04$; df = 8; $p < 0.025$; Fig. 2). In the nestling stage, Little Galloo Island exhibited differences among sampling periods ($G = 45.49$; df = 20; $p < 0.001$), but there were no differences at Birnie Rocks ($G = 20.50$; df = 16; $p > 0.10$) or Bustard Islands ($G = 4.62$; df = 8; $p > 0.75$).

At Little Galloo, Pigeon, and Snake islands, alewife occurred most often in boli (87, 85, and 88%, respectively; Appendix 3). When the occurrence of alewife and other fish items was compared at Lake Ontario colonies among sampling periods, no differences were found at Little Galloo Island ($G = 5.91$; df = 3; $p > 0.10$; Fig. 3) or Pigeon Island ($G = 3.09$; df = 4; $p > 0.50$). At Snake Island, nestling diet composition varied between 21 and 29 June ($G = 6.86$; df = 1; $p < 0.01$); alewife increased from 80 to 92% occurrence in boli, whereas other fish items decreased from 56 to 12%. At Bustard Islands, a decrease in the percent occurrence of alewife (70–33%) and rainbow smelt (36–6%) accounted for most of the difference in diet composition between sampling dates ($G = 12.72$; df = 3; $p < 0.01$; Fig. 3).

Spatial variation

During the egg stage on Lake Ontario, there was an overall difference in food items occurring in pellets among Little Galloo, Pigeon, and Snake islands ($G = 190.4$; df = 10; $p < 0.001$; Fig. 4, Appendix 2). However, there was no difference between Pigeon and Snake islands ($G = 1.06$; df = 2; $p > 0.50$). Alewife occurred more frequently in pellets from Little Galloo Island (45%) than Pigeon Island (4%) or Snake Island (4%). Centrarchids and yellow perch also occurred much less frequently at Little Galloo Island. On Lake Huron, there were also differences in diet composition between Birnie Rocks and Bustard Islands during the egg stage ($G = 22.06$; df = 8; $p < 0.005$) and nestling stage ($G = 27.04$; df = 8; $p < 0.001$) (Fig. 1, Appendix 2).

The diet composition of nestlings appeared to be homogeneous among Little Galloo, Pigeon, and Snake islands ($G = 2.40$; df = 2; $p > 0.25$; Fig. 5, Appendix 3). All three colonies on Lake Ontario were highly dependent on a single prey item (alewife). In contrast, on Lake Huron, there were differences in nestling diet composition among all four study colonies ($G = 143.9$; df = 12; $p < 0.001$; Fig. 5, Appendix 3). The only identified species at Manitoba Reef was alewife, occurring in 88% of boli. Centrarchids (46.8%) were the most common diet item at Gull Rock, whereas at Birnie Rocks and Bustard Islands nestling diet was less dominated by a single food item (Fig. 5, Appendix 3).

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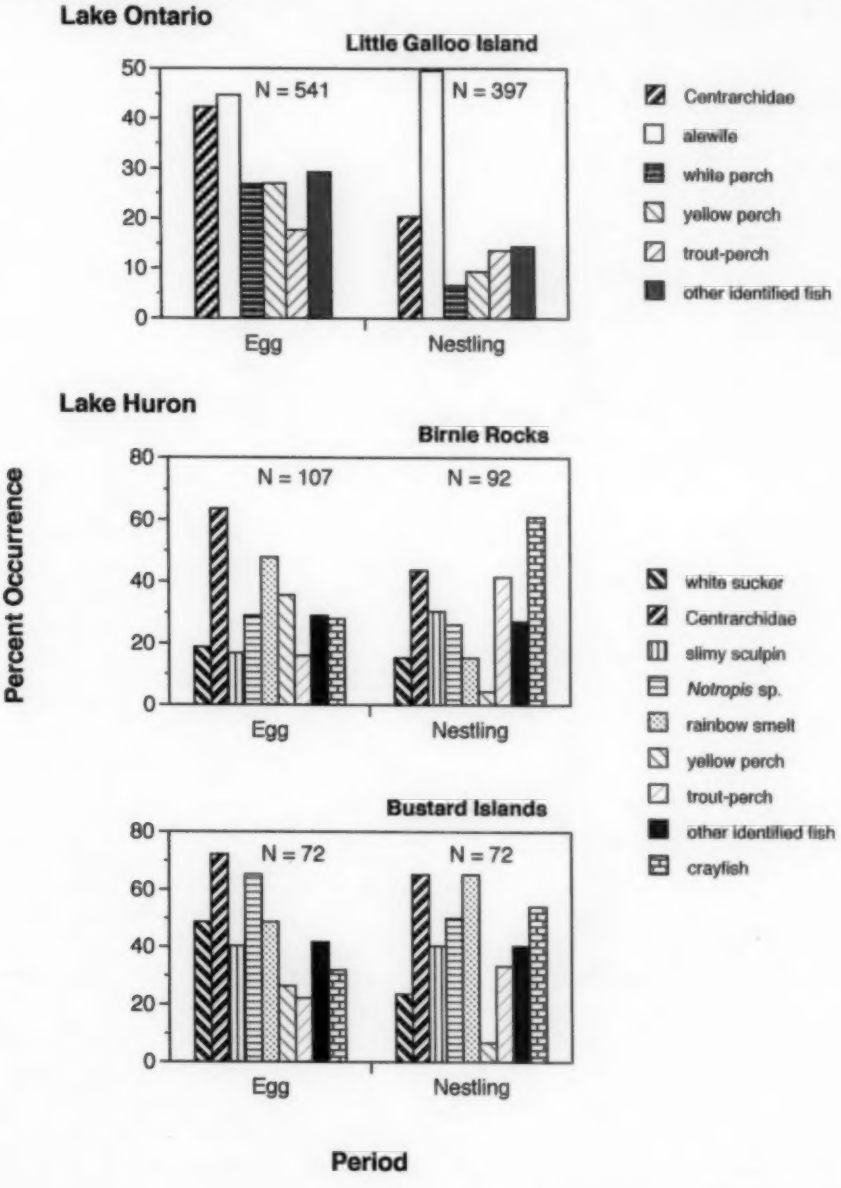
Fig. 1. Variation in double-crested cormorant diet composition between the egg and nestling stage of three colonies, based on percent frequency of occurrence of food items in (N) pellet samples.



Pellets and boli

To compare diet composition of nestlings and adults, given the temporal and spatial variation we detected, we examined differences in the contents of pellets and boli when both sampling date and location were the same (Table 3). For the two colonies and three dates having suitable data for these comparisons, boli generally contained about 30% fewer fish families. Although some fish types occurred in broadly similar frequencies in pellets and boli, others showed large differences. White sucker were common in pellets at Birnie Rocks (21%) and Bustard Islands (22 and 25%); however, they were never identified in these boli. Gasterosteidae were common in boli but rarely occurred in pellets. Trout-perch, centrarchids, and crayfish occurred commonly in pellets but were much rarer in boli.

Prey length

In June 1992, the median length of intact alewife regurgitated by nestlings varied little among the three Lake Ontario colonies studied (range and sample size are given in parentheses): Little Galloo Island, 14.6 cm (5.1–17.1, $n = 87$ fish); Pigeon Island, 14.1 cm (7.7–17.3, 48); Snake Island, 14.7 cm (7.2–16.5, 33). The only other colony for which prey lengths

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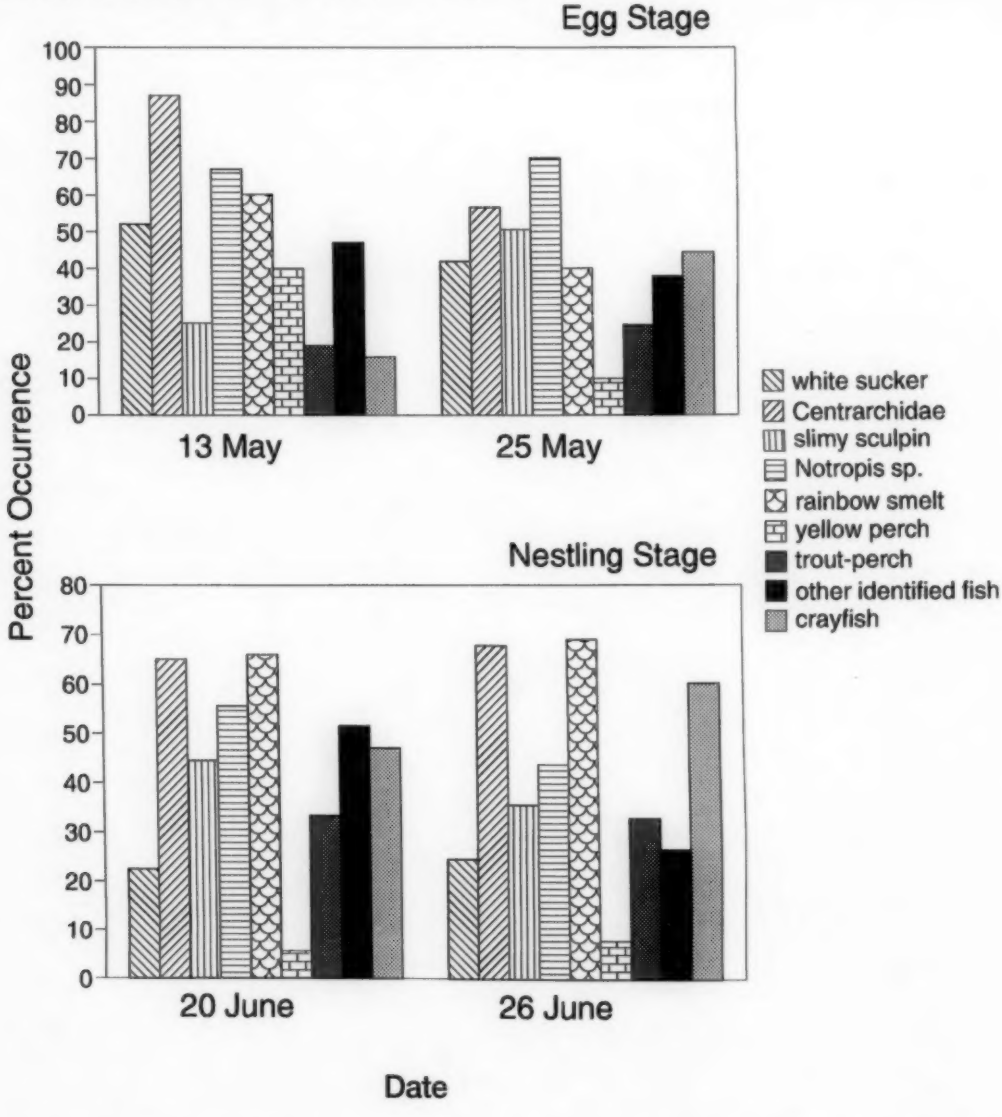
Fig. 2. Temporal variation in diet composition of double-crested cormorants within the egg and nestling stage, at Bustard Islands, Lake Huron, 1994, based on percent frequency of occurrence of food items in (N) pellets.



were recorded was East Sister Island, Lake Erie, on 23 June 1994, when median lengths of regurgitated fish varied widely according to species: emerald shiner, 7.6 cm (5.1–10.2, n = 214 fish); white perch, 19.1 cm (12.7–21.6, 21); yellow perch, 17.2 cm (14.0–19.1, 4); walleye, 30.5 cm (10.2–35.6, 17); freshwater drum, 22.9 cm (15.2–30.5, 9).

Discussion

The results of this, the first extensive study on the diet of double-crested cormorants on the lower Great Lakes, indicate the importance of spatial and temporal factors in understanding the diet of double-crested cormorants. As a result, the temporal and spatial scales at which biologists and fishery managers have previously investigated the effect of double-crested cormorant predation on fish communities may introduce considerable bias and error. Therefore, we would recommend that sampling be conducted at a scale appropriate to the question being posed. For example, an assessment of local impacts of cormorant predation should focus on that area only, ensuring that temporal variation is determined. Extrapolations to a whole-lake context can only be made meaningfully once spatial and temporal variability has been quantified.

Temporal variation

We found significant differences in the proportion of major food items in pellets between the egg and nestling stage. These differences may be attributed to the movement of certain fish

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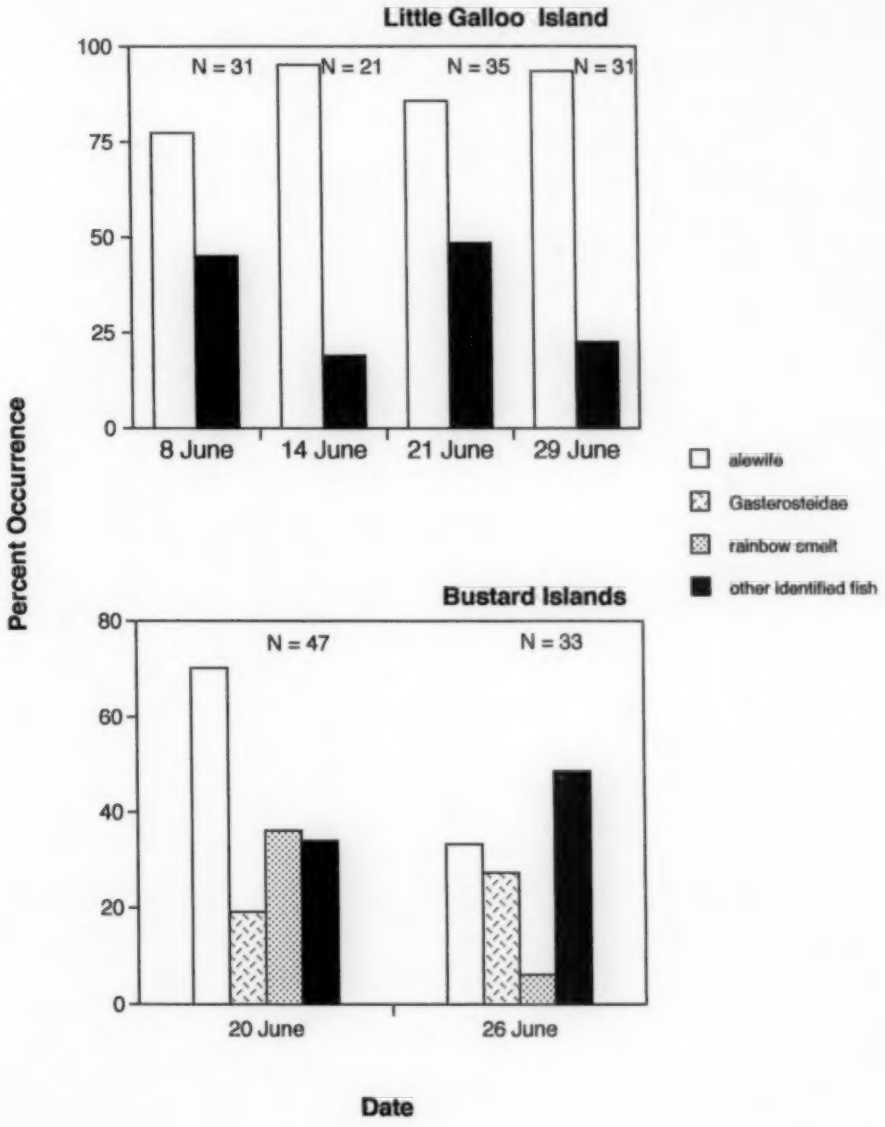
Fig. 3. Temporal variation in diet composition of nestling double-crested cormorants, based on percent frequency of occurrence of fish types in (N) boli.



species into shallower spawning habitats that make them more vulnerable to cormorant predation. For instance, yellow perch, a littoral zone species, moves into the shallows of lakes and tributary rivers to spawn early in spring (Scott and Crossman 1973) and then moves into deeper waters when water temperatures reach 15°C (Whiteside et al. 1985); this species showed large decreases in percent occurrence in pellets from colonies on Lake Ontario and Lake Huron between the two stages (Fig. 1). There were significant differences in the percent occurrence of prey items between sampling dates within the egg stage at Little Galloo Island, Birnie Rocks, and Bustard Islands (Fig. 2). However, at Birnie Rocks and Bustard Islands, there were no significant differences between sampling dates during the nestling stage. This suggests that following fish spawning in spring, the prey selection for double-crested cormorants becomes more stable. At Little Galloo Island this relationship did not hold, suggesting that this type of stability may vary with location and prey availability. Changes in fish behaviour during development (Brown 1985; Post and McQueen 1988) will make certain age-classes more vulnerable to predation during different periods of the cormorant breeding season. Unfortunately, this type of age-class information is difficult to obtain from diet data collected from pellets, especially when differential digestion rates of different-sized otoliths exists (e.g., Johnstone et al. 1990).

The diet of chicks based on boli was always dominated by

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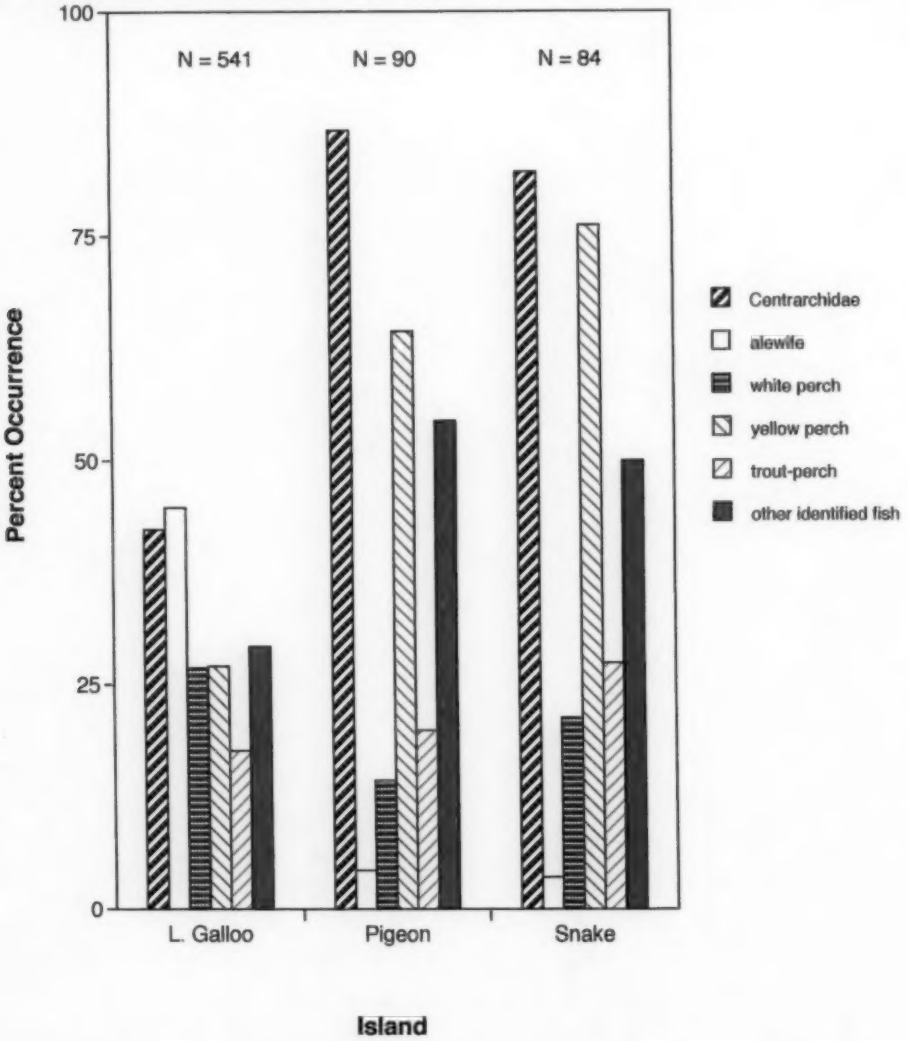
Fig. 4. Spatial variation in diet composition of double-crested cormorants during the egg stage, 1992, among three colonies in eastern Lake Ontario, based on percent frequency of occurrence of fish types in (N) pellets.



relatively small, forage species (Appendix 3). On Lake Ontario, the diet of chicks was dominated by a single, relatively high-energy species, alewife (Rottiers and Tucker 1982). Alewife had the greatest occurrence in boli during all sampling dates, and significant differences among sampling dates at Snake Island were due to a change in the percent occurrence of a lumped category of non-alewife species (including unidentifiable fish), rather than indicating any biologically significant event. At Bustard Islands on Lake Huron, there were significant changes in the proportion of forage species that were found in boli among sampling dates (Fig. 3). These forage species are usually found in large schools (Scott and Crossman 1973) and are often encountered by cormorants foraging in large flocks (Glanville 1992; Orta 1992). Differences between sampling dates at Bustard Islands are probably explained by random variation in cormorants' encounters with these schools (Ainley et al. 1981; Duffy et al. 1987).

Blackwell et al. (1995) reported no significant temporal variation in diet composition of nestling double-crested cormorants in Penobscot Bay, Maine, but their sampling was restricted to only two stages during the 1993 nestling period. Interestingly, their results do indicate marked temporal shifts in the longer term in availability of different prey species, with groundfish (notably Atlantic cod, *Gadus morhua*, and flounders) being very scarce in cormorant diets in 1993, compared with the situation in the 1930s (Mendall 1936). Cormorants can clearly serve as good indicators of relative abundance of fish species (e.g., Linn and Campbell 1992; Warke and Day 1995).

Spatial variation

Several studies have shown that within a lake, there is a great deal of spatial heterogeneity of fish assemblages resulting from variations in habitat structure (Eadie and Keast 1984; Benson and Magnuson 1992; Leslie and Timmins 1992, 1994;

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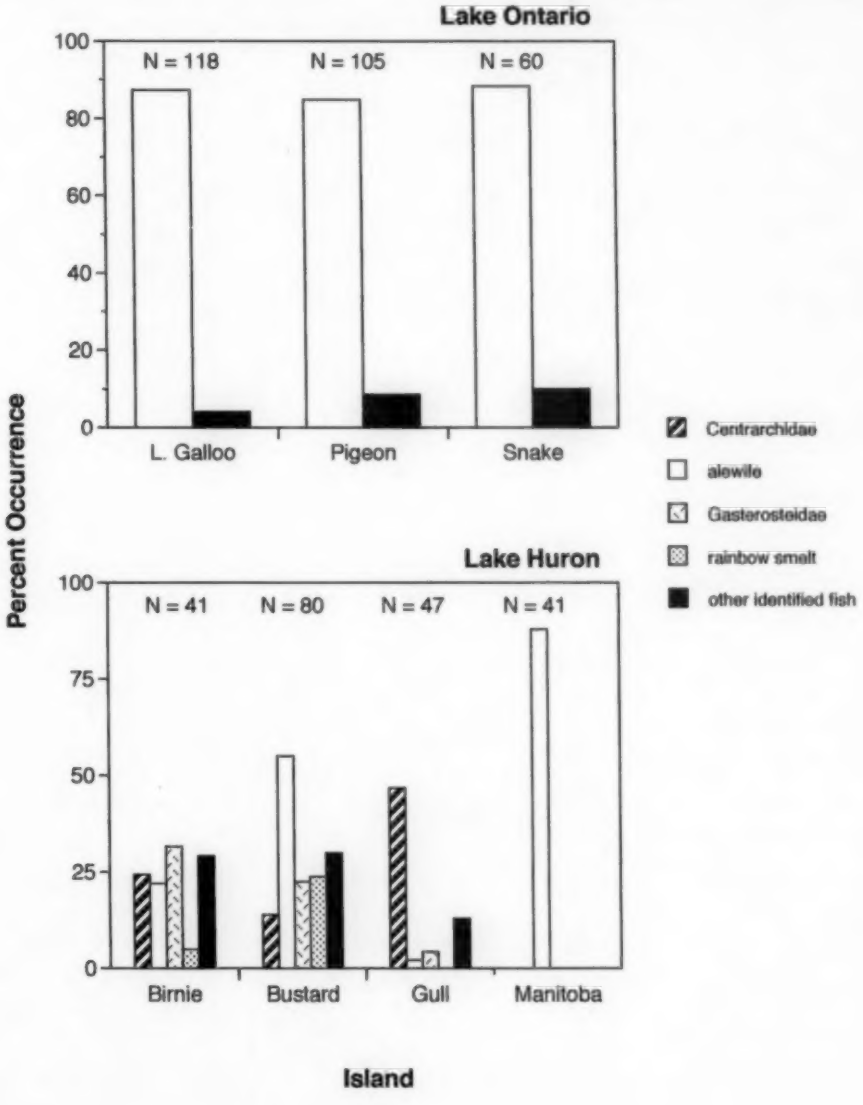
Fig. 5. Spatial variation in diet composition of nestling double-crested cormorants, based on percent frequency of occurrence of fish types in (N) boli.



Spangler and Collins 1992). While breeding cormorants may travel as much as 40 km to a foraging site, in Wisconsin they usually travelled no further than 3 km (Custer and Bunck 1992). Our observations at Canadian Great Lakes' colonies suggest that many birds forage within approximately 10 km of their breeding colony. At Little Galloo Island, cormorants foraged on average 13 km (range 3.7–20 km) from the colony (K. Karwowski, unpublished data). As a result, the location of a breeding colony can have a significant effect on the food items found in boli and pellets.

Differences in bathymetry surrounding breeding colonies on Lake Huron seem to be consistent with the significant differences in chick diet among colonies. Gull Rock is found in the North Channel of Lake Huron in relatively shallow waters, while Manitoba Reef is located in more open, deeper waters. This could explain why the boli from Manitoba Reef were dominated by alewife, an open-water species during the cormorant nestling period, while centrarchids, found in shallower waters of the littoral zone, occurred most frequently in boli at Gull Rock (Fig. 5). Birnie Rocks and Bustard Islands are offshore islands surrounded by water of various depths, including access to shallow nearshore areas and river mouths; the broader range of prey items in boli at these colonies (Fig. 5) is consistent with access to a wide variety of fish habitats. However, boli were only sampled once at Gull Rock and Manitoba Reef, and based on data from pellets at these colonies, a similar relationship between bathymetry and adult diet is absent (Appendix 2).

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Table 3. Differences in percent frequency of occurrence of food items in pellets (P) and boli (B) collected on the same date from two colonies in Georgian Bay, Lake Huron.

	Birnie, 17 June 1994		Bustard			
			20 June 1994		26 June 1994	
Food item	Pellet ($n = 28$)	Bolus ($n = 26$)	Pellet ($n = 36$)	Bolus ($n = 47$)	Pellet ($n = 36$)	Bolus ($n = 33$)
Fish items						
Catostomidae						
Catostomus commersoni	21.4	—	22.2	—	25.0	—
Centrarchidae						
Ambloplites rupestris	10.7	15.4	19.4	6.4	8.3	12.1
Lepomis sp.	7.1	—	—	—	5.6	—
Micropterus dolomieu	17.9	7.7	33.3	4.3	16.7	6.1
Unknown sp.	21.4	—	38.9	2.1	58.3	—
Clupeidae						
Alosa pseudoharengus	10.7	23.1	27.8	70.2	19.4	33.3
Cottidae						
Cottus cognatus	17.9	7.7	44.4	6.4	36.1	24.2
Cyprinidae						
Notropis hudsonius	—	—	—	8.5	—	—
Notropis sp.	25.0	—	55.6	4.3	44.4	—
Esocidae						
Esox lucius	3.6	—	2.8	—	—	—
Gasterosteidae						
Apeltes quadracus	—	—	—	2.1	—	—
Gasterosteus aculeatus	—	3.8	—	2.1	—	3.0
Pungitius pungitius	—	23.1	2.8	19.2	—	21.2
Unknown species	—	19.2	—	—	—	6.1
Ictaluridae						
Ameiurus nebulosus	3.6	—	5.6	—	2.8	—
Osmeridae						
Osmerus mordax	17.9	3.8	61.1	36.2	69.4	6.1
Percichthyidae						
Morone americana	—	3.8	—	—	—	—
Percidae						
Etheostoma nigrum	—	—	—	2.1	—	—
Etheostoma sp.	—	7.7	—	—	—	—
Perca flavescens	10.7	—	5.6	12.8	8.3	9.1
Perca caprodes	—	—	—	2.1	—	—
Stizostedion vitreum	21.4	—	22.2	—	11.1	—
Percopsidae						
Percopsis omiscomaycus	14.3	3.8	33.3	4.3	33.3	3.0
Salmonidae						
Coregonus clupeaformis	—	—	—	—	2.8	—
Coregonus artedi	3.6	—	—	—	2.8	—
Unknown fish species	35.7	46.2	88.9	68.1	94.4	60.6
No. of identified fish species	12	9	12	13	12	9
No. of identified fish families	11	8	11	8	10	7
Non-fish items						
Bird (egg shell)	—	—	2.8	2.1	—	—
Arthropoda						
Crayfish	46.4	—	47.2	—	61.1	3.0
Insect	10.7	—	27.8	—	38.9	—
Snail	7.1	—	19.4	—	13.9	—

Because of the temporal variation we demonstrated, we stress that any conclusions about spatial variation in diet should be made with caution, especially when conclusions are based on a limited number of sampling dates. Even where there is similar bathymetry, such as at Birnie Rocks and Bustard Islands, there were differences between the colonies in the occurrence of prey items in pellets and boli during the more temporally stable nestling stage (Figs. 1 and 5). This is not

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surprising considering that fish assemblages can be quite variable even over short distances along a shoreline (e.g., Benson and Magnuson 1992).

On Lake Ontario, there was no spatial difference in the diet of juveniles, based on boli (Fig. 5). However, during the egg stage, adult diet composition varied significantly between Little Galloo Island and the other islands studied. These differences occurred over a small scale relative to the size of the lake and are unlikely to be explained by temporal factors, considering the large number of samples and sampling dates (Table 1). Snake Island's location very close to the mainland and other islands offers access to extensive areas of shallow waters and bays within 5 km. On the other hand, Little Galloo Island is approximately 8 km from the mainland and is surrounded by deeper water. This is consistent with Snake Island having a larger occurrence of shallow, warmwater fish like centrarchids and yellow perch, while alewife appeared more frequently in the pellets from Little Galloo Island (Scott and Crossman 1973). However, the diet from Pigeon Island, which is approximately 6 km from the nearest large land mass and surrounded by deeper water, showed no significant difference from that at Snake Island. The effect of competition and (or) prey depletion on the availability of certain prey species may be an important factor considering that Little Galloo Island has about 10 times the number of occupied nests compared with Snake and Pigeon islands (Weseloh et al. 1995). The energetic costs of reaching different foraging areas and differences in the fish community structure are other likely factors that contribute to spatial variation in diet among these sites (Eadie and Keast 1984; Alcock 1989).

Comparisons of nestling diet composition among three groups of colonies in Maine, in a 25 km long stretch of Penobscot Bay, found no significant spatial variation in a 1993 study, but numbers of boli collected and colonies studied were considerably smaller than in our study (Blackwell et al. 1995).

Pellets versus boli

While studying pellets offers a nondestructive way to determine diet in adult cormorants, certain biases should be recognized. Analysing pellet contents does not differentiate between food items consumed directly, as opposed to remains of items contained within fish stomachs (i.e., 'secondary consumption'). Although we know of no studies that have been able to quantify this bias, we suspect that it occurred in our samples and was probably large for crayfish exoskeleton fragments and otoliths of very small fish found in pellets. In controlled feeding studies, it is clear that otoliths from different fish species are not equally recoverable from pellets because of differences in digestibility (Duffy and Laurenson 1983; Johnstone et al. 1990; Brugger 1993). In fact, the egestion of a pellet may depend on the species of fish eaten (Brugger 1993), as well as the size and combination of fish types ingested (P.J. Ewins, personal observation). Unfortunately, little is known about the effect of quality, quantity, and diversity of prey species consumed on the proportion of otoliths recovered in a pellet (Johnstone et al. 1990). Cormorants often produce fewer or more than one pellet per day; therefore, a single pellet should not be taken to represent 1 day's intake of food (Duffy and Laurenson 1983; Johnstone et al. 1990; Russell et al. 1995). However, many of these biases would be more problematic if we were attempting to make comparisons based on relative abundance or conclusions on the complete diet of double-crested cormorants (Barrett et al. 1990).

Johnson and Ross (1996) have demonstrated recently that, for adult double-crested cormorants breeding at the Little Galloo Island colony, analysis of fecal material provided only 4% additional diagnostic fish remains beyond those indentified from pellets. At present, pellets seem to provide the most cost-effective, representative, and non-intrusive means of assessing diet composition of adult cormorants.

In comparing the diet of adults and chicks, it is important to recognize the difference in the egestion material used to make these comparisons. Biases associated with these different types of egested food remains are regarded as very important for a wide range of aquatic bird species, with pellets generally over-representing food items with hard, indigestible structures (e.g., Duffy and Jackson 1986; Johnstone et al. 1990; Brown and Ewins 1996). A cormorant pellet provides information about prey items consumed over a period of one or more days, but a bolus represents the contents of a recent meal. As a consequence, our comparisons of prey items found in boli and pellets on the same day may not provide a useful control for temporal factors (Table 3); the larger number of fish families found in pellets compared with boli may only reflect the larger number of meals represented in a pellet. However, relatively small, forage species constitute the main fish groups in boli, suggesting that adults may be selecting smaller prey items when foraging for their young (Appendix 3). Lengths of intact fish we found in boli from young cormorants were usually within the range for these species caught by commercial fishermen. Although our fish size data were from very few dates and locations, we assume that they provide a good indication of the size of prey items fed to chicks. However, because we did not examine digestive tracts of foraging adults, nor estimate prey sizes from the length of otoliths found in pellets because of biases from differential digestion and abrasion (Duffy and Laurenson 1983; Johnstone et al. 1990; Brugger 1993), we are unable to quantify the sizes of prey consumed by adult cormorants.

Sampling design

Limited sampling both spatially and temporally may provide misleading information about cormorant diets. Other detailed studies have documented a great deal of spatial and temporal heterogeneity in the diet of piscivorous waterbird species even within a limited area (Duffy et al. 1987; Barrett et al. 1990; Ewins et al. 1994). On the lower Great Lakes the only accounts of the diet of double-crested cormorants are from a single colony, Little Galloo Island (Karwowski et al. 1994; Ross and Johnson 1995; Johnson and Ross 1996). While bioenergetic models have demonstrated that double-crested cormorants do not pose a significant threat to piscivorous fish species within an entire lake (Weseloh and Casselman 1992; Madenjian and Gabrey 1995), there have been few studies on the local effects of heavy predation. If cormorants from a colony were concentrating their foraging efforts on selected species during certain periods, such as the spawning stage, they may have biologically significant effects on fish survival and recruitment. Such potentially strong impacts might well be overlooked if cormorant diet samples were not collected at appropriate times and places. In fact, evidence exists for double-crested cormorants depleting prey biomass around marine colonies (Birt et al.

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1987). This may be particularly important in disturbed areas where spawning and nursery habitat has become scarce due to anthropogenic effects (Leslie and Timmins 1992).

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Appendix 1. Fish species identified in pellets and boli of double-crested cormorants.

Catostomus commersoni	White sucker
Ambloplites rupestris	Rock bass
Lepomis gibbosus	Pumpkinseed
Lepomis macrochirus	Bluegill sunfish
Micropterus dolomieu	Smallmouth bass
Micropterus salmoides	Largemouth bass
Pomoxis nigromaculatus	Black crappie
Alosa pseudoharengus	Alewife
Dorosoma cepedianum	Gizzard shad
Cottus cognatus	Slimy sculpin
Luxilus cornutus	Common shiner
Cyprinus carpio	Carp
Notemigonus crysoleucas	Golden shiner
Notropis atherinoides	Emerald shiner
Notropis hudsonius	Spottail shiner
Rhinichthys cataractae	Longnose dace
Esox lucius	Northern pike

Appendix 1 (*concluded*).

Apeltes quadracus	Four-spine stickleback
Gasterosteus aculeatus	Three-spine stickleback
Pungitius pungitius	Nine-spine stickleback
Amerius nebulosus	Brown bullhead
Ictalurus punctatus	Channel catfish
Noturus flavus	Stonecat
Osmerus mordax	Rainbow smelt
Morone americana	White perch
Etheostoma nigrum	Johnny darter
Perca flavescens	Yellow perch
Percina caprodes	Log perch
Stizostedion vitreum	Walleye
Percopsis omiscomaycus	Trout-perch
Coregonus clupeaformis	Lake whitefish
Coregonus artedii	Lake herring
Aplodinotus grunniens	Freshwater drum
Umbra limi	Central mudminnow

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Appendix 2. Percent frequency of occurrence of food items in pellets of double-crested cormorants collected during the egg stage (E), nestling state (N), and total (Tot) at study colonies in eastern Lake Ontario (1992) and Lake Huron (1994).

	Lake Ontario							Lake Huron										
	Galloo			Pigeon		Snake		Birnie			Bustard			Gull		Manitoba Reef		
Food item	E, 541	N, 397	Tot, 938	E, 90	Tot, 107	E, 84	Tot, 90	E, 107	N, 92	Tot, 199	E, 72	N, 72	Tot, 144	E, 32	Tot, 35	E, 36	N, 46	Tot, 82
Fish items																		
Catostomidae																		
Catostomus commersoni	1.3	1.0	1.2	16.7	15.9	11.9	12.2	18.7	15.2	17.1	48.6	23.6	36.1	12.5	11.4	19.4	15.2	17.1
Centrarchidae																		
Ambloplites rupestris	1.3	0.8	1.1	8.9	12.2	8.3	8.9	1.9	5.4	3.5	5.6	13.9	9.7	6.3	8.6	—	2.2	1.2
Lepomis gibbosus	14.8	2.5	9.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lepomis macrochirus	5.7	2.0	4.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lepomis sp.	—	—	—	26.7	29.0	32.1	31.1	16.8	4.3	11.1	15.3	2.8	9.0	21.9	22.9	—	—	—
Micropterus dolomieu	7.2	5.3	6.4	44.4	43.9	42.9	45.6	55.1	14.1	36.2	56.9	25.0	41.0	87.5	85.7	25.0	2.2	12.2
Micropterus salmoides	0.4	—	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pomoxis nigromaculatus	0.7	—	0.4	1.1	0.9	1.2	1.1	—	—	—	1.4	—	0.7	—	—	—	—	—
Unknown sp.	24.2	13.9	19.8	86.7	86.9	82.1	83.3	15.0	30.4	22.1	41.7	48.8	45.1	40.6	42.9	13.9	13.0	13.4
Clupeidae																		
Alosa pseudoharengus	44.7	49.6	46.8	4.4	10.3	3.6	4.4	5.6	9.8	7.5	1.4	23.6	12.5	6.3	5.7	—	19.6	11.0
Dorosoma cepedianum	—	0.3	0.1	—	—	1.2	1.1	—	—	—	—	—	—	—	—	—	—	—
Cottidae																		
Cottus cognatus	6.5	2.5	4.8	15.6	14.0	14.3	13.3	16.8	30.4	23.1	40.3	40.3	40.3	15.6	14.3	27.8	41.3	35.4
Cyprinidae																		
Luxilus cornutus	0.4	—	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Notemigonus crysoleucas	0.7	—	0.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Notropis hudsonius	0.9	0.8	0.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Notropis sp.	10.4	8.1	9.4	25.6	24.3	14.3	14.4	29.0	26.1	27.6	65.3	50.0	57.6	62.5	57.1	36.1	19.6	26.8
Unknown sp.	—	—	—	—	—	—	—	0.9	1.1	1.0	1.4	—	0.7	—	—	—	2.2	1.2
Esocidae																		
Esox lucius	1.8	0.3	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Esox sp.	0.2	—	0.1	—	—	—	—	—	1.1	0.5	—	1.4	0.7	—	—	—	—	—
Gasterosteidae																		
Gasterosteus aculeatus	—	—	—	—	—	—	—	0.9	—	0.5	—	—	—	—	—	—	—	—
Pungitius pungitius	—	—	—	—	—	—	—	0.9	—	0.5	1.4	1.4	1.4	—	—	—	—	—
Unknown sp.	—	—	—	—	—	—	—	0.9	—	0.5	—	—	—	—	—	—	—	—
Ictaluridae																		
Ameiurus nebulosus	5.4	1.0	3.5	18.9	18.7	6.0	5.6	15.9	4.3	10.6	19.4	4.2	11.8	—	2.9	5.6	8.7	7.3
Ictalurus punctatus	—	—	—	—	—	1.2	1.1	—	—	—	—	—	—	—	—	—	—	—
Noturus flavus	0.2	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Osmeridae																		
Osmerus mordax	5.5	2.0	4.1	12.2	11.2	22.6	22.2	47.7	15.2	32.7	48.6	65.3	56.9	40.6	40.0	36.1	30.4	32.9
Percichthyidae																		
Morone americana	26.8	6.5	18.2	14.4	15.9	21.4	21.1	—	—	—	—	—	—	—	—	—	—	—
Percidae																		
Perca flavescens	27.0	9.3	19.5	64.4	64.5	76.2	77.8	35.5	4.3	21.1	26.4	6.9	16.7	53.1	51.4	25.0	8.7	15.9
Stizostedion vitreum	2.6	0.5	1.7	—	—	—	—	2.8	13.0	7.5	6.9	16.7	11.8	21.9	20.0	44.4	34.8	39.0
Percopsidae																		
Percopsis omiscomaycus	17.7	13.6	16.0	20.0	22.4	27.4	31.1	15.9	41.3	27.6	22.2	33.3	27.8	12.5	14.3	2.8	8.7	6.1
Petromyzontidae	0.2	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Salmonidae																		
Coregonus clupeaformis	0.6	—	0.3	—	—	—	1.1	—	—	—	—	1.4	0.7	—	—	—	—	—
Coregonus artedi	—	—	—	3.3	2.8	2.4	2.2	5.6	1.1	3.5	13.9	1.4	7.6	21.9	20.0	19.4	17.4	18.3
Salmoninae sp.	—	—	—	—	—	1.2	1.1	—	—	—	—	—	—	—	—	—	—	—
Unknown sp.	2.2	—	1.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sciaenidae																		
Aplodinotus grunniens	—	—	—	—	—	1.2	1.1	—	—	—	—	—	—	—	—	—	—	—
Unknown fish sp.	20.1	20.7	20.4	57.8	56.1	46.4	50.0	70.1	46.7	59.3	83.3	91.7	87.5	65.6	62.9	58.3	67.4	63.4
No. of identified fish species	22	16	23	12	12	15	16	13	11	13	13	13	14	10	11	9	11	11
No. of identified fish families	13	11	13	11	11	12	12	11	11	12	11	12	12	9	10	9	10	10

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Appendix 2 (*concluded*).

	Lake Ontario							Lake Huron										
	Galloo			Pigeon		Snake		Birnie			Bustard			Gull		Manitoba Reef		
Food item	E, 541	N, 397	Tot, 938	E, 90	Tot, 107	E, 84	Tot, 90	E, 107	N, 92	Tot, 199	E, 72	N, 72	Tot, 144	E, 32	Tot, 35	E, 36	N, 46	Tot, 82
Non-fish items																		
Bird (egg shell/feather)	—	—	—	18.9	18.7	4.8	6.7	—	—	—	—	1.4	0.7	—	—	—	—	—
Amphibian[a]	2.0	0.5	1.4	5.6	4.7	14.3	14.4	—	—	—	—	—	—	—	—	—	—	—
Arthropoda																		
Crayfish	na	na	30.1	38.9	42.1	34.5	33.3	28.0	60.9	43.2	31.9	54.2	43.1	34.4	37.1	25.0	17.4	20.7
Insect[b]	—	—	—	2.2	3.7	—	—	6.5	30.4	17.6	2.8	33.3	18.1	3.1	2.9	8.3	32.6	22.0
Mollusca[c]	na	na	8.7	33.3	35.5	33.3	33.3	11.2	12.0	11.6	19.4	16.7	18.1	6.3	8.6	5.6	13.0	9.8
Other invertebrate	0.9	0.8	0.9	4.4	3.7	3.6	3.3	4.7	2.2	3.5	—	—	—	—	—	—	2.2	1.2
Vegetation	na	na	21.4	56.7	54.2	52.4	50.0	—	—	—	—	—	—	—	—	—	—	—

[a]Includes mudpuppy, *Necturus maculosus*.
[b]Terrestrial and aquatic species.
[c]Gastropod snails and bivalve molluscs.

Appendix 3. Percent of frequency of occurrence of food items in boli produced by nestling double-crested cormorants at study colonies in eastern Lake Ontario (1992), Lake Huron and Lake Erie (1994).

	Lake Ontario			Lake Huron				Lake Erie,
Food item	Galloo (118)	Pigeon (105)	Snake (60)	Birnie (41)	Bustard (80)	Gull (47)	Man (41)	E. Sister (69)
Fish items								
Catostomidae								
Catostomus commersoni	—	—	—	—	—	21	—	—
Centrarchidae								
Ambloplites rupestris	0.8	1.9	3.3	19.5	8.8	38.3	—	2.9
Micropterus dolomieu	0.8	1.9	—	4.9	5.0	4.3	—	—
Unknown sp.	1.7	5.7	3.3	—	1.3	6.4	—	—
Clupeidae								
Alosa pseudoharengus	87.3	84.8	88.3	22.0	55.0	2.1	87.8	—
Cottidae								
Cottus cognatus	—	—	—	9.8	13.8	2.1	—	—
Cyprinidae								
Cyprinus carpio	—	—	—	—	—	—	—	1.4
Notropis atherinoides	—	—	—	—	—	—	—	50.7
Notropis hudsonius	1.7	1.9	—	—	5.0	—	—	—
Notropis sp.	—	—	—	4.9	2.5	—	—	—
Rhinichthys cataractae	—	—	—	—	—	2.1	—	—
Gasterosteidae								
Apeltes quadracus	—	—	—	—	1.3	—	—	—
Gasterosteus aculeatus	—	—	—	4.9	2.5	2.1	—	—
Pungitius pungitius	—	—	—	19.5	20.0	2.1	—	—
Unkown sp.	—	—	—	14.6	2.5	2.1	—	—
Ictaluridae								
Ameiurus nebulosus	—	—	—	—	—	2.1	—	—
Ictalurus punctatus	—	—	—	—	—	—	—	1.4
Osmeridae								
Osmerus mordax	—	—	—	4.9	23.8	—	—	—
Percichthyidae								
Morone americana	—	—	—	2.4	—	—	—	24.6
Percidae								
Etheostoma nigrum	—	—	—	—	1.3	—	—	—
Etheostoma sp.	—	—	—	4.9	—	—	—	—
Perca flavescens	1.7	1.0	6.7	2.4	11.3	4.3	—	5.8
Percina caprodes	—	—	—	—	1.3	—	—	—
Stizostedion vitreum	—	—	—	—	—	—	—	21.7

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Appendix 3 (*concluded*).

Food item	Lake Ontario			Lake Huron				Lake Erie,
	Galloo (118)	Pigeon (105)	Snake (60)	Birnie (41)	Bustard (80)	Gull (47)	Man (41)	E. Sister (69)
Percopsidae								
Percopsis omiscomaycus	—	—	—	4.9	3.8	—	—	—
Sciaenidae								
Aplodinotus grunniens	—	—	—	—	—	—	—	13.0
Umbridae								
Umbra limi	—	—	—	2.4	—	—	—	—
Unknown fish sp.	32.2	43.8	21.7	46.3	65.0	46.8	51.2	1.4
No. of identified fish species	5	5	3	11	13	10	1	8
No. of identified fish families	4	4	3	10	8	8	1	6
Non-fish items								
Bird (egg shell)	—	—	1.7	—	1.3	—	—	—
Crayfish	0.8	—	—	2.4	1.3	6.4	2.4	—
Snail	—	1.0	—	—	—	—	—	—
Vegetation	7.6	11.4	3.3	—	—	—	—	—

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Individual variation in dispersal behaviour of newly emerged chinook salmon (*Oncorhynchus tshawytscha*) from the Upper Fraser River, British Columbia

Michael J. Bradford and Garth C. Taylor

Abstract: Immediately after emergence from spawning gravels, fry of stream-type chinook salmon (*Oncorhynchus tshawytscha*) populations from tributaries of the upper Fraser River, British Columbia, distribute themselves downstream from the spawning areas, throughout the natal stream, and into the Fraser River. We tested the hypothesis that this range in dispersal distances is caused by innate differences in nocturnal migratory tendency among individuals. Using an experimental stream channel, we found repeatable differences in downstream movement behaviour among newly emerged chinook fry. Fish that moved downstream were larger than those that held position in the channel. However, the incidence of downstream movement behaviours decreased over the first 2 weeks after emergence. We propose that the variation among individuals in downstream movement behaviour we observed leads to the dispersal of newly emerged fry throughout all available rearing habitats. Thus, between- and within-population variation in the freshwater life history observed in these populations may be caused by small differences in the behaviour of individuals.

Résumé : Immédiatement après l'émergence des lits de gravier des frayères, les alevins de saumon chinook de cours d'eau (*Oncorhynchus tshawytscha*) appartenant aux populations des tributaires du cours supérieur du fleuve Fraser, en Colombie-Britannique, se dispersent en aval des aires de frais, dans l'ensemble du cours d'eau natal et dans le fleuve Fraser. Nous avons vérifié l'hypothèse que cette gamme de distances de dispersion soit attribuable à des différences innées dans les tendances migratoires nocturnes chez les individus. En utilisant un chenal de cours d'eau expérimental, nous avons observé des différences répétées dans le comportement de déplacement en aval chez les alevins de saumon chinook nouvellement émergés. Les poissons qui se sont déplacés vers l'aval étaient de taille plus grande que ceux qui sont demeurés sur place dans le chenal. Cependant, l'incidence des comportements de déplacement vers l'aval a diminué au cours des deux premières semaines suivant l'émergence. Nous estimons que la variation observée entre les individus pour ce qui est du comportement de déplacement vers l'aval mène à la dispersion des alevins dans la totalité des habitats d'alevinage disponibles. Ainsi, la variation interpopulations et intrapopulation observée dans le cycle vital d'eau douce dans ces populations peut être attribuable à de petites différences dans le comportement des individus.
[Traduit par la Rédaction]

Introduction

The Fraser River is the single largest producer of chinook salmon (*Oncorhynchus tshawytscha*) on the Canadian Pacific coast, with recent escapements in the order of 100 000 – 200 000 spawners (Northcote and Larkin 1989). One population spawns in the upper reaches of the Fraser mainstem, and some 40–50 populations utilize nearly every large tributary along its length. Except for some southern tributaries, most populations have the stream-type life history, where juveniles rear in freshwater for a year before they migrate to the sea (Gilbert 1913; Fraser et al. 1982).

A quantitative understanding of the freshwater life history of the stream-type populations is lacking and this paucity of information makes it difficult to identify factors that might limit production or to predict the impacts of habitat change (e.g., Bradford 1994). However, some basic information on the seasonal migrations of juveniles are available. Immediately after emergence from the spawning gravel in April and May, many fry disperse downstream, largely at night (Shepherd et al. 1986). Fry can move downstream as far as 100 km within a few days of emergence (M.J. Bradford and G.C. Taylor, unpublished data). By June, juveniles are distributed throughout the natal stream (e.g., Murray et al. 1981; Taylor et al. 1995), the Fraser mainstem (Allan et al. 1995), as well as in small non-natal tributaries that drain into the mainstem (Murray and Rosenau 1989; Scrivener et al. 1994). After this initial dispersal, most fish take up feeding positions, although trapping studies in natal streams indicate that small numbers of juveniles continue to move downstream throughout the year (Levings and Lauzier 1989; Taylor et al. 1994). Nearly all juveniles emigrate to the ocean as age-1 smolts the following spring.

The mechanism that causes some individuals to remain in their natal areas while others migrate long distances from spawning areas to the Fraser mainstem after emergence is unknown (Healey 1991). One possibility is that all fry have

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essentially the same behaviour and that their distribution is the result of chance events. Fry emergence is usually coincident with the spring–summer freshets, and a proportion of emerging fish could be swept downstream by strong currents. Others may find refuge in habitat features along the margins of the stream, and these fish may form the group that rears near the spawning areas.

The alternative hypothesis is that fish actively disperse downstream, and the propensity to disperse varies among individuals. Some fish may not move at all, some may disperse only initially after emergence, while others may move for a longer period, resulting in dispersal a great distance from the spawning area.

The objective of this study is to describe the ontogeny of migratory behaviour of stream-type chinook fry during their first 3 weeks after emergence. Our experiment is an elaboration of earlier work on the rheotactic behaviour published by Taylor (1988, 1990). Taylor found movement patterns of chinook juveniles observed in the laboratory during the first year of life agreed with field observations of migratory behaviour. He observed that stream-type juvenile chinook salmon tended to move downstream at night during the first 3 months following emergence, but afterwards this negative rheotaxis subsided. Negative rheotaxis increased in 1-year-old juveniles (Taylor 1990), which corresponds to the timing of seaward migration of smolts observed in the field. Taylor did not, however, explicitly examine variation in movement patterns during the first few days after emergence nor did he investigate individual variation in behaviour. The role of individual variation in both life history evolution and population dynamics is being increasingly recognized (e.g., McLaughlin and Grant 1994; Tyler and Rose 1994).

Materials and methods

We indexed nocturnal downstream movement behaviour by placing fry in an experimental stream channel at dusk and recording the distance individuals moved after a fixed time interval (see Kelso et al. 1980; Taylor 1988 for other examples). We repeated this procedure four times over 3 weeks, and by using a system of marks on the fish, we were able to document the behaviour of individual fish over the whole experiment.

Newly emerged chinook fry were collected from four upper Fraser River chinook populations during the peak of the outmigration in mid-May 1995. The populations were from the following streams (with the distance from the main spawning area to the Fraser mainstem and the 1994 brood year escapement in parentheses): Bowron River (150 km, 9100), Dome Creek (2 km, 530), Fraser River at Tete Jaune (0 km, 4200) and Slim Creek (50 km, 2500). Locations of the streams are indicated on Fig. 1 of Bradford (1994), with the exception of Dome Creek, which is 5 km southeast of Slim Creek. Fry were captured from each system with an inclined plane trap (IPT) located immediately below the main spawning area. We assumed that fry caught in the IPTs were very recently emerged due to the close proximity of the traps to the spawning areas and the uniform, small size of the fry captured. There were at least 200 redds upstream of the traps, so it is likely that the fry collected were a mixture of a number of families. At each location, a trap was installed in the evening and left overnight. From the catch, approximately 100 fry were transported by truck to the experiment site at Purden Lake, 60 km southeast of Prince George, B.C. Transportation time from each spawning area was less than 2 h. Fry were held outdoors in plastic containers (40 × 23 × 20 cm) placed in fibreglass flow-through rearing troughs. Screened openings in the containers allowed water to flow through at a rate of about 0.9 $L \cdot min^{-1}$. The four populations were held separately for the duration of the experiment and fed once daily with flaked fish food.

The experiment was performed at the Purden Lake site using four experimental channels similar in design to those of Northcote and Kelso (1981). Each channel was 4.8 m in length and divided into 14 equal-sized compartments (33 × 30 × 30 cm) numbered from 1 to 14 (upstream to downstream). A 3.8 cm diameter hole was drilled through each compartment divider, staggered in the configuration described by Northcote and Kelso (1981). Fish moving between compartments were thus required to "seek out" the openings and could not move directly upstream or downstream. Water was supplied from a small, unnamed creek to both the rearing troughs and the experimental channels through a single head tank. Water flow was maintained at 2.75 $L \cdot min^{-1}$ in each channel producing a velocity of 12 $cm \cdot s^{-1}$ through the interconnecting holes. Within each compartment water moved slowly in a circular pattern. Water temperatures in the channels rose gradually from 5 to 9°C during the experiment, similar to the pattern observed in the natal streams (e.g., Allan et al. 1995).

Trials were run at 2, 7, 13, and 22 days after the day of capture of the experimental fish. To start a trial, fry from a single population were divided into 4 groups of 25, and each group was placed in compartment 2 of a replicate trough 1 h before civil twilight (Nautical Almanac Office 1995; civil twilight is about 45 min after sunset). Only 66 fry were captured from Dome Creek, so they were divided equally among three troughs. Wire screens covered the holes in the compartment 2 dividers to prevent fry from leaving during the acclimatization period. At civil twilight, the screens were removed from the openings allowing the fry access to all compartments. Forty-five minutes later the water flow was stopped and the opening between compartments 5 and 6 was blocked to prevent further movement of fry. The total number of fry in each compartment was counted with the aid of a flashlight.

We classified fry as "stayers" if they were found in the upper half of the trough (i.e., compartments 1–7) at the end of the experiment, or "movers" if they moved downstream to compartments 8–14. Movers and stayers were dipnetted out of the experimental troughs and held separately until the following morning for marking. We used fin marks or fin clips to mark fry as either movers or stayers after each trial, so that we could follow the behavioural history of each fish. Following the first trial, stayers were marked on the caudal fin with India ink from a Panjet dental inoculator (Hart and Pitcher 1969). Movers were injected with water as a sham treatment. Fry were similarly marked on the anal fin after the second trial and an adipose fin clip was used to mark movers after the third trial. Fish were anaesthetized with 2-phenoxyethanol before marking, and the fork length was recorded. After marking, all fish from the trial were returned to a single holding container. We kept all fish from each population in a single container and allocated them randomly to the experimental troughs for the trials to minimize the effects that social hierarchies might have on migration behaviour. For the Slim population, 13 fish were accidentally killed after trial 3, and two Bowron and a Fraser fish were lost during the experiment. Following the last trial, fry were preserved in 50% isopropanol. The marks on each fish were then decoded, and each fish was placed in 1 of 16 categories based on its behaviour over the four trials.

Statistical analyses

Movement patterns

We analysed differences in the movement of fry among the four populations in two ways. The first method used the counts of fish scored as either stayers or movers in 2 × 4 contingency tables to test for heterogeneity among the four populations in the proportions in the two behaviour categories. The G test (Sokal and Rohlf 1995, p. 738) was used for this analysis. For

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the second method, movement scores were calculated using the method of Kelso et al. (1980), where each fish was assigned a value from 1 to 14 depending on which compartment it was found in at the end of the trial. A mean score was calculated for each trough, and a one-way ANOVA was conducted on the means, with values from the three or four troughs serving as replicates. Tukey's test (SAS Institute Inc. 1988) was used to make post hoc comparisons of movement scores among populations. Because we used the same fish throughout the experiment, analyses of data from the different trials are not completely independent of each other, and significance levels may be inflated. We could not use a repeated measures analysis because the distribution of individuals in the replicate troughs varied randomly among trials.

Temporal changes in movement for each population were analysed by Cochran's Q test (Sokal and Rohlf 1995, p. 781), which is appropriate when individuals are repeatedly scored for an attribute over time. For each population we used the pattern of marks on each fish to reconstruct the behaviour of individuals over the four trials and used these data to test for heterogeneity in behaviour over time.

Analysis of individual behaviours

We modelled the change in the behaviour of individuals between trials with 2×2 transition matrices. Entries in each matrix were the proportion of fish of jth behaviour (moving or staying) in trial $k + 1$ that had the ith behaviour in trial k. Matrix elements $p_{1,1}$ and $p_{2,2}$ were the proportions of fish that remained as stayers or movers, respectively, in both trials, $p_{1,2}$ was the proportion that changed from moving to staying and $p_{2,1}$ was the fraction of stayers in the first trial that became movers in the second. The columns of the matrix sum to 1, and each matrix has a characteristic vector that is the stable distribution among behaviours that the population will converge to if the original population vector is repeatedly multiplied by the matrix, i.e., $\boldsymbol{t}_k = \boldsymbol{A}^k \boldsymbol{t}_0$, where $\boldsymbol{A}$ is the transition matrix and $\boldsymbol{t}_k$ is a 2×1 vector containing the stable numbers (or proportions) of movers and stayers. Homogeneity among transition matrices, either over time or among populations was tested with Anderson and Goodman's (1957) test statistic, which compares the elements in each matrix to a matrix constructed by pooling the data (see Usher (1979) for applications of this test, as well as further discussion of transition matrices).

A test of independence was used to determine if the behaviour in one trial was influenced by behaviours in the previous trial. This test would indicate, for example, whether fish that moved in one trial were more likely to be classed as movers in subsequent trials. We used the procedure of Anderson and Goodman (1957), as described by Usher (1979, p. 416), which is essentially the same as a standard G test for independence (Sokal and Rohlf 1995, p. 731). Kendall's $\tau - b$ coefficient was used to measure the degree of association between behaviours in different trials (SAS Institute Inc. 1988).

Body size and movement

The relation between dispersal and fish length was estimated for each trial with logistic regression that included fork length, population, and length × population terms as independent variables. As before, analyses for the four trials are not independent of each other because they were based on the same total population of fish. However, individuals in each movement category varied from trial to trial as fish changed behaviour categories over time; this would tend to diminish the non-independence of the data across trials.

Results

Variation in movement among populations

When fish were first introduced to the experimental channel at sunset they swam into the current and held position. At twilight, when the trial began, some fish sought out the openings between partitions and began moving downstream (facing downstream) through the compartments, while others were relatively quiescent and held their position. At the end of the trials the distribution of chinook juveniles in the stream channels was often U shaped (Fig. 1), with some individuals grouped near the point of introduction, others in the bottom two compartments, and the remainder distributed amongst the middle compartments. The U-shaped distributions supports our hypothesis of variation in dispersal behaviour among individuals of each population.

In the first trial, conducted 2 days after capture, the majority of Bowron and Slim fish moved to the downstream compartments and were classified as movers (Figs. 1 and 2). Fraser and Dome results were more dichotomous, as some fish remained in the compartments near the point of introduction, while others moved to the downstream end (Fig. 2). Differences among populations were confirmed by both the G test ($G = 39.1$, $P < 0.0001$, df = 3) and the ANOVA on mean scores ($F_{3,11} = 8.26$, $P = 0.004$). Tukey's test indicated that the scores of the Bowron and Slim fish were higher than those from the Fraser and Dome populations ($P < 0.05$).

There were no consistent differences in movement scores in the last three trials (Fig. 2). Heterogeneity was observed in the third trial ($G = 23.7$, $P < 0.0001$, df = 3; ANOVA: $F_{3,11} = 5.26$, $P = 0.017$), and Tukey's test on the mean scores indicated that significance was due to high movement scores for the Slim population (Fig. 2). No differences among populations were observed for the second and forth trials (all $P > 0.3$).

Overall, the proportion of fish classified as movers decreased over time, although there was a slight increase in the last trial (Fig. 2). Changes in movement patterns over time were significant (Cochran's Q test: Bowron, $Q = 39.4$; Dome, $Q = 37.7$; Fraser, $Q = 88.8$; Slim, $Q = 90.2$; all $P < 0.0001$).

Individual variation in behaviour

Transition matrices for the change in behaviour of individuals from the first to the second trial were similar among populations (Anderson and Goodman's (1957) test, $\chi^2_6 = 3.2$, $P = 0.8$). We combined the data from the four populations and calculated an average matrix for this interval (Table 1). The elements of the matrix indicate that over 80% of fish that were stayers in the first trial remained as stayers for trial 2. Of movers, slightly more than half switched to becoming stayers in trial 2, while the rest remained as movers (Table 1).

We found heterogeneity in transition matrices among populations for the interval between the second and third trials ($\chi^2_6 = 31.1$, $P < 0.001$) caused by the greater than expected change of stayers to movers for the Slim population. This anomaly resulted in the high movement score for the Slim

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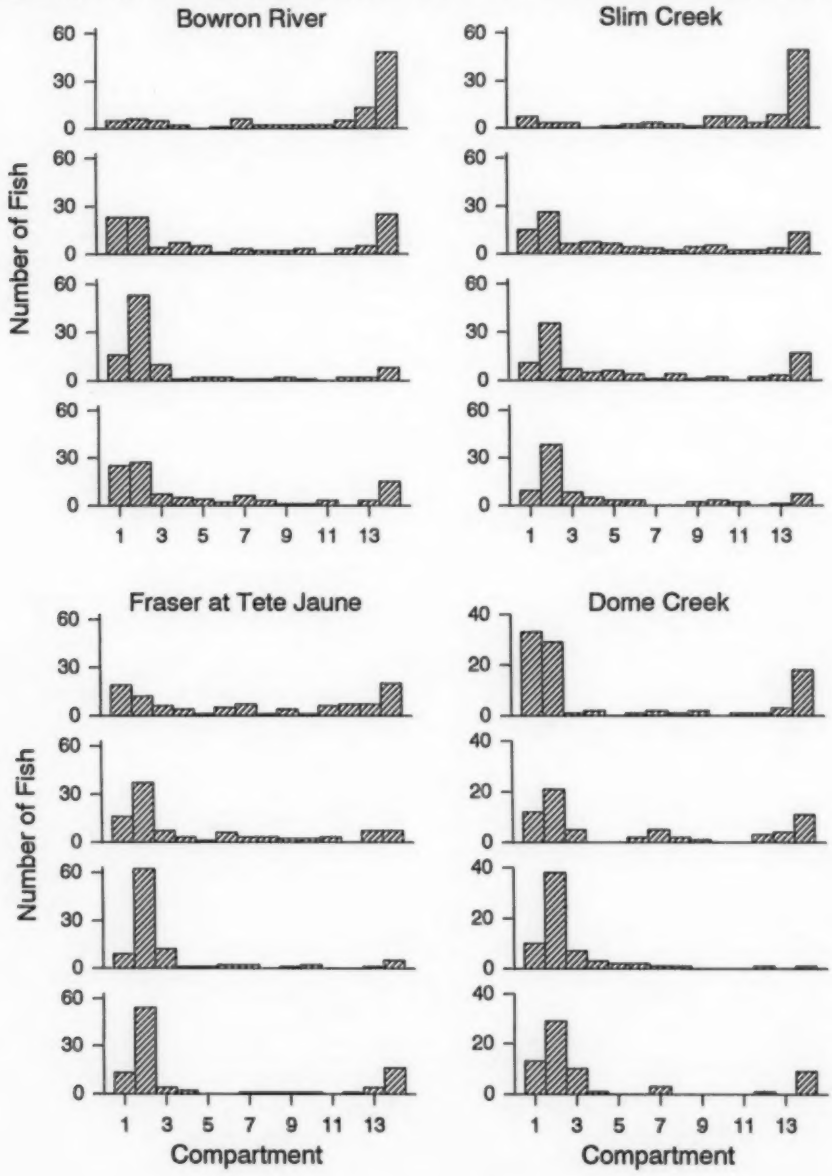
Fig. 1. The location of fish in the stream channels at the end of each trial for four study populations. Fish were introduced in chamber 2 and could move upstream to compartment 1 or downstream towards chamber 14 at the bottom of the channel. For each population, histograms are results from trials 1 (top) through 4 (bottom). For each population, data are summed over four replicate troughs (but three replicates for Dome).



population in trial 3 (Fig. 2). No differences were found for the last interval ($\chi^2_6 = 9.6$, $P = 0.07$). We ignored the heterogeneity in the second interval and calculated transition matrices for each time interval using movement data summed over all populations. There was some evidence for differences among these matrices ($\chi^2_4 = 10.5$, $P = 0.03$, Table 1). The stable population vectors calculated from the three matrices were comprised of 72–87% stayers and 13–28% movers (Table 1). The projections of change in behaviour for trials 2–4 calculated from transition matrices closely mapped the observed changes in behaviour over the experiment (Fig. 2, broken lines).

The behaviour of an individual in a trial was influenced by its behaviour in previous trials, indicating there were repeatable differences in movement tendency among individuals. We rejected the null hypothesis of independence of behaviours in the transition matrices (all populations combined), for the intervals between successive trials (for the interval between trials 1 and 2: $\chi^2_1 = 22$; trials 2 and 3: $\chi^2_1 = 13$; trials 3 and 4: $\chi^2_1 = 31$, all $P < 0.0001$). The $\tau - b$ coefficients ranged from

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Fig. 2. The proportion of fish scored as movers (i.e., found in compartments 8–14 in Fig. 1) at the end of each trial. The broken lines are results of the projections of the transition matrices of Table 1. Results from the first trial were used as the initial vector for these projections; data were combined into two groups: Bowron–Slim and Dome–Fraser.

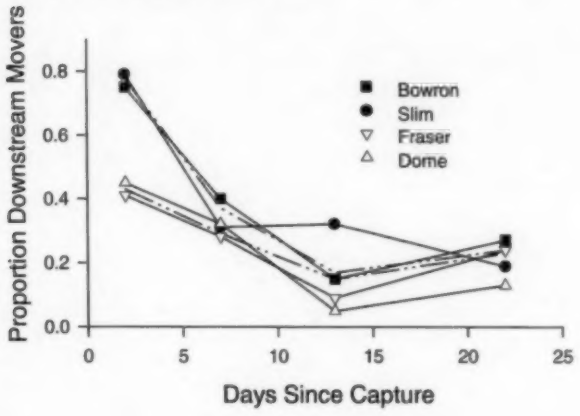

Table 1. Transition matrices for the change in the proportion of movers (M) and stayers (S) from trial k to $k+1$ for the intervals between successive the trials, averaged over the four populations.

	Interval between trials								
	1–2			2–3			3–4		
	S_k	M_k	t_{1-2}	S_k	M_k	t_{2-3}	S_k	M_k	t_{3-4}
S_{k+1}	0.81	0.58	75	0.89	0.74	87	0.83	0.42	72
M_{k+1}	0.19	0.42	25	0.11	0.26	13	0.17	0.58	28

Note: The 2×1 vectors t contain the stable percentages of stayers and movers that would result from the repeated application of the transition matrices.

0.20 to 0.33, indicating that individuals tended to retain the same behaviour from one trial to the next. We also found a similar dependence between behaviours separated by two trials (interval between trials 1 and 3: $\chi^2_1 = 12$, $P < 0.001$, $\tau - b = 0.18$; trials 2 and 4 $\chi^2_1 = 22$, $P < 0.001$, $\tau - b = 0.26$).

Further insight into the ontogeny of movement behaviours can be gained by an examination of the sequence of behaviours of individuals. Most fish in the experiment (257 of 346, 74%) were stayers or movers through all trials or were movers initially and switched to become stayers for the remainder of the experiment (Table 2). Thus, the predominate ontogenetic sequence could be described as a switch from moving to staying behaviour. For some individuals, the switch was never observed, while for others it occurred during the 3 weeks of the experiment. The remaining fish (26% of the total) switched from staying to moving (opposite to the majority) at least once during the experiment. There was no difference among populations in the distribution of fish among the four categories of Table 2 ($G = 5.2$, $P = 0.16$, df = 3).

Movement and body size

Movement behaviour was significantly correlated with fish length in the first three trials, and a weak effect was observed

Table 2. Counts of fish for each of 16 possible outcomes of the four trials.

		Population				
Category	Sequence	Bowron	Dome	Fraser	Slim	Total
Predominant	SSSS	14	30	39	13	96
	MSSS	30	13	20	34	97
	MMSS	16	6	8	12	42
	MMMS	5	0	0	6	11
	MMMM	4	2	2	3	11
3S 1M	SMSS	4	6	7	0	17
	SSMS	0	0	1	0	1
	SSSM	4	1	4	3	12
3M 1S	SMMM	1	0	2	0	3
	MSMM	2	0	3	4	9
	MMSM	8	5	7	4	24
2M 2S	SSMM	0	0	1	1	2
	SMMS	0	1	0	0	1
	SMSM	1	1	1	1	4
	MSMS	3	0	0	2	5
	MSSM	6	1	4	0	11

Note: The sequence column shows the behaviours over trials 1–4, as indicated by the letters (M, movers; S, stayers). Category labelled predominate constitutes fish that were either movers or stayers in all four trials or were initially movers and then changed to become stayers for the remainder of the experiment. Other categories are those that switched from staying to moving behaviour and are grouped depending on the distribution of the two behaviours.

Table 3. Mean length of stayers (S) and movers (M) at each trial.

		Population			
Trial	Behaviour	Bowron	Dome	Fraser	Slim
1	S	36.7 (0.27)	36.2 (0.30)	36.4 (0.22)	36.1 (0.26)
	M	36.7 (0.20)	37.0 (0.34)	36.5 (0.23)	37.2 (0.22)
2	S	36.9 (0.18)	36.7 (0.27)	37.0 (0.20)	37.2 (0.15)
	M	36.7 (0.24)	38.0 (0.40)	37.7 (0.30)	37.4 (0.21)
3	S	37.2 (0.16)	37.0 (0.22)	37.2 (0.18)	37.3 (0.19)
	M	37.8 (0.36)	38.3 (0.88)	39.3 (0.69)	37.9 (0.22)
4	S	38.3 (0.18)	38.3 (0.27)	38.6 (0.24)	38.2 (0.20)
	M	37.8 (0.40)	39.2 (0.61)	39.3 (0.43)	39.0 (0.37)

Note: Values are the mean fork lengths in mm with SE given in parentheses; N varies from 3 to 89.

for the fourth (logistic regression, trial 1: $\chi^2_1 = 7.1$, $P = 0.008$; trial 2: $\chi^2_1 = 5.8$, $P = 0.016$; trial 3: $\chi^2_1 = 12.6$, $P = 0.0004$; trial 4: $\chi^2_1 = 3.2$, $P = 0.074$). For all trials the population and length × population effects were not significant (all $P > 0.10$). Movers were longer than stayers, although the difference in mean length was often 1 mm or less (Table 3).

Discussion

The main findings of our study support our hypothesis that there is intra- and inter-populational variation in downstream movement behaviour of newly emerged chinook fry that could contribute to the range of dispersal distances observed in the field. Within each population, individual chinook salmon fry varied in their tendency to move downstream, and for most fish, movement behaviour followed a predictable ontogenetic sequence. Our experimental data do not support the hypothesis

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that the dispersal of chinook fry after emergence is the result of chance interactions between individuals possessing a single phenotype and the environment. Rather, the bimodal distribution of movements in some trials, the repeatability of behaviour, and the differences among populations suggest there is considerable phenotypic variability among individuals in their tendency to move downstream.

Relating our results to the behaviour of fish in the field depends on the assumption that the movement behaviours we observed index the actual migratory tendency of newly emerged fry. We cannot test this assumption directly but note that Taylor (1988, 1990) found agreement between rheotactic behaviour measured in the laboratory and field data for chinook populations of differing life histories. The experimental design we used was successful in providing an index of behaviour for other salmonids (Raleigh 1971; Northcote and Kelso 1981; Brannon 1972). Further, we describe below how our experimental results for Slim Creek fish are in agreement with recent field data on the downstream migrations of chinook fry in this system. We will therefore discuss our results on the premise that our experimental results are correlated with migratory behaviour in the field.

Other researchers have suggested that salmonid fry have a flow-sensitive period in their early ontogeny when they are sensitive to being swept downstream by strong currents (e.g., Ottaway and Forrest 1983; Irvine 1986). Our results suggest our fish had behaviour analogous to a sensitive period in the first 2 weeks after capture, when most downstream movements occurred. However, trapping studies show that over 90% of upper Fraser fry migrate at night (e.g., Taylor et al. 1994), indicating that downstream movements during this sensitive period are not passive as nearly all fish are able to find refuge and avoid being swept away by the current during the day. Fish classified as movers in our experiment might be those fish that enter the current at night and are carried downstream. Individuals that oriented themselves to the current during our trials and were classed as stayers might be those that maintain their positions during the night and will form the group that rear in the stream near spawning areas. Undoubtedly, some of the stayers are probably displaced downstream by the high flows that occur during spring months.

More fish from the longer streams (Slim and Bowron) moved downstream in the first trials, and this result raises the hypothesis that the proportion of newly emerged chinook fry that actively migrate downstream might be greater for populations of longer tributaries than for shorter streams. Field studies indicate a large proportion of newly emerged fry from the two longer tributary streams undergo lengthy migrations, with many reaching the Fraser mainstem soon after emergence (Taylor et al. 1994; G.C. Taylor and M.J. Bradford, unpublished data). Fish from these populations had the highest movement scores in the first trial. Fry from Dome Creek had lower movement scores in the first trial than was observed for the two longer systems; however, spawning in Dome Creek occurs 1–6 km upstream from the Fraser River, and little active migration would be necessary to disperse the fry population to the Fraser mainstem. Similar low scores were observed for fry from Fraser mainstem population.

Differences among populations in movement disappeared with 7 days of capture, and there was a large drop in dispersal behaviours over the first three trials. Reimers (1971) also observed that the propensity of newly emerged fall chinook fry from the Sixes River, Oregon, to move downstream decreased during the first 4 days after emergence. In the case of Slim Creek, marking studies indicate that fry can travel downstream from the main spawning area at a rate of up to 25–40 km per night and thus need only 2–3 days to reach the Fraser mainstem (M.J. Bradford and G.C. Taylor, unpublished data). In the larger Bowron system, higher water velocities during the May freshet (peak discharges in the lower Bowron range from 300 to 400 $m^3 \cdot s^{-1}$ compared with about 50 $m^3 \cdot s^{-1}$ for Slim Creek) and a less complex river channel probably assist in carrying fry downstream at a faster rate. Thus, downstream migratory propensity may only be necessary for the first few days after emergence to disperse the population considerable distances downstream from the spawning areas. However, we cannot rule out the possibility that the decrease in dispersal behaviour we observed in our experiment resulted from holding the fish between trials in chambers that permitted limited volitional movements. The moving phenotype was still present in each population at the end of the experiment, which is consistent with field studies that show a continuous small downstream dispersal of chinook juveniles throughout the year (e.g., Levings and Lauzier 1989; Taylor et al. 1994).

Larger fish were more likely to move downstream than were smaller ones. This tendency was observed for the first trial, 2 days after capture; variation in fish size at this age is most likely due to differences established prior to emergence, rather than through growth from exogenous feeding. Egg size can play a major role in emergent fry size in chinook salmon (Fowler 1972; Withler et al. 1987), although other factors such as stream temperature and intragravel conditions will also be important (Brannon 1965). If egg size is correlated with the size (and age) of female spawners, then the offspring of larger (older) females will tend to be larger at emergence and may disperse further downstream those of smaller females.

Our finding that larger fish were more likely to move argues against the hypothesis that downstream movements in our trials were caused by hierarchical interactions among individuals. For coho salmon (*O. kisutch*), migrant fry are usually smaller than resident fish, and this is thought to be due to the displacement of smaller fish by socially dominant larger individuals (Chapman 1962; Mason 1975; Hartman et al. 1982); similar results were described for chinook fry rearing in a small stream channel (Reimers 1968). If downstream movement was stimulated by behavioural interactions, then we would expect fish to distribute themselves evenly among the compartments of the stream channel rather than tending to be clumped at each end. Further, we would expect interactions to increase as fry grow older and larger, but in the later trials we found fish were even more concentrated. Unfortunately, for our study populations, the importance of social interactions among individuals in the field during the first few days after emergence is unknown. During the peak of emergence, large numbers of fry swim from the gravel each night and take up temporary residence along the stream margins during the day. At this time of year, freshet flows typically flood marginal habitats with turbid, cold water, and it is not clear that under these conditions social hierarchies would have an opportunity to develop. Social interactions are probably more likely later in the season when flows decrease and stabilize and water clarity improves.

In conclusion, our analysis has indicated considerable

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individual variation in the behaviour of newly emerged chinook salmon fry from populations of upper Fraser River region. We propose that differences in rheotactic behaviour in the first week after emergence, coupled with exposure to strong river currents, leads to the dispersal of fry from spawning areas to the Fraser mainstem. The proportion of the year-class that migrates long distances from the natal area may depend on the innate propensity of individuals to move downstream, their size, and the morphology and hydrology of the natal stream. Thus the early life history of upper Fraser River chinook may vary considerably from stream to stream, and this variation will present particularly difficult challenges to those attempting to identify the factors or habitats that limit or regulate the productivity of these populations.

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Cyprinid distributions in Northeast U.S.A. lakes: evidence of regional-scale minnow biodiversity losses

Thomas R. Whittier, David B. Halliwell, and Steven G. Paulsen

Abstract: Fish assemblages were sampled in 195 randomly selected lakes in the northeastern United States during the summers of 1991–1994. Most lakes in northern Maine had three to seven minnow species, constituting 40–80% of species in each lake. Lakes in New Jersey, southern New York, and southern New England rarely had minnows, other than golden shiner (*Notemigonus crysoleucas*). Lakes in the Adirondacks and the remainder of northern New England had intermediate numbers. We examined minnow native ranges and autecology and evaluated species richness related to littoral predators and human disturbance. Sample data suggested alteration in the minnow assemblages over much of the region. The most consistent factor related to minnow species richness was the presence of littoral predators. Median number of minnow species was two in lakes lacking predators and zero in lakes with predators. Non-native predators, especially *Micropterus* spp., have been introduced throughout the Northeast; 69% of the sampled lakes had non-native predators. In the absence of predators, minnow species declined with increased human activity in the watershed and along lake shorelines. Only in northern Maine did lake minnow assemblages seem relatively intact.

Résumé : Les assemblages de poissons ont été échantillonnés dans 195 lacs choisis au hasard dans le nord-est des États-Unis au cours des étés 1991–1994. La plupart des lacs situés dans le nord du Maine comptaient trois à sept espèces de cyprins, constituant 40–80 % des espèces dans chacun des lacs. Les lacs du New Jersey, du sud de l'État de New York et du sud de la Nouvelle-Angleterre comptaient rarement des cyprins autres que la chatte de l'est (*Notemigonus crysoleucas*). Les lacs des Adirondacks et du reste du nord de la Nouvelle-Angleterre comptaient, quant à eux, un nombre intermédiaire de cyprins. Nous avons examiné les aires de répartition naturelle et l'autécologie des cyprins et évalué la richesse en espèces en fonction des prédateurs du littoral et des perturbations causées par l'activité humaine. Les données de l'échantillonnage indiquent une modification des assemblages de cyprins dans une grande partie de la région. Le facteur le plus constant lié à la richesse en espèces de cyprins était la présence de prédateurs littoraux. Le nombre médian d'espèces de cyprins dans les lacs n'abritant pas de prédateurs était de deux, alors qu'il était de zéro dans les lacs comptant des prédateurs. Des prédateurs non indigènes, surtout des espèces du genre *Micropterus*, ont été introduits partout dans le nord-est; 69 % des lacs échantillonnés comptaient des prédateurs non indigènes. En l'absence de prédateurs, la richesse en espèces de cyprins diminuait en fonction de l'augmentation de l'activité humaine dans le bassin hydrographique et le long du rivage du lac. Les assemblages de cyprins ne semblaient relativement intacts que dans les lacs situés dans le nord du Maine.
[Traduit par la Rédaction]

Introduction

There is growing concern with loss of aquatic biodiversity (Williams et al. 1989; Hughes and Noss 1992; Winter and Hughes 1995). Minnows (Cyprinidae) are the most diverse freshwater fish family, with over 1700 species worldwide and over 200 in North America (Page and Burr 1991; Winfield and Nelson 1991). Yet, minnows are among the fishes most sensitive to chemical, biological, and habitat perturbations, which in turn, make minnows susceptible to local, watershed, and regional extirpation. For example, in Virginia, minnows constitute 7 of the 26 fish species extirpated from at least one major drainage (Angermeier 1995). In Adirondack lakes, Baker et al. (1990, 1993) listed 13 of 18 minnow species as acid sensitive, a proportion similar to that found in Ontario lakes by Matuszek et al. (1990). In northern Wisconsin, Tonn and Magnuson (1982) found that minnows were generally absent or present in very low numbers in lakes that supported littoral piscivores, which are the species most often introduced to improve fishing.

Local species losses, coupled with widespread introductions of successful non-native fishes, lead to a reduction in β diversity, a regional-scale homogenization of fish assemblages (Radomski and Goeman 1995). Local extinction events often go unnoticed, until the regional or global situation becomes critical (Angermeier 1995). Unfortunately, the broad-scale view needed to detect regional patterns of local species losses is rarely available. The detailed reports of state-wide whole

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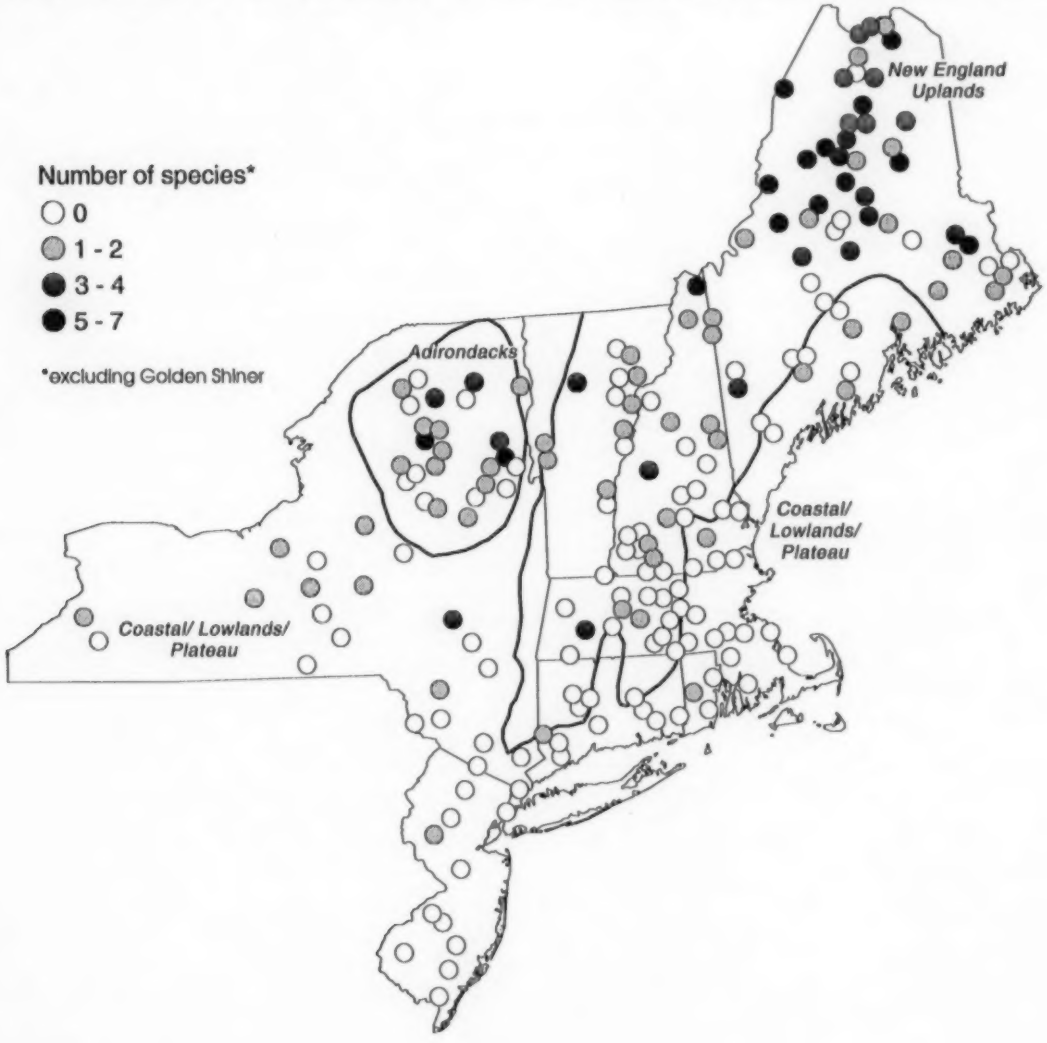
Fig. 1. Native minnow species richness (excluding golden shiner) at 195 lakes sampled by EMAP for fish during the summers of 1991–1994. Lines show three subregions for lakes in the northeastern United States; areas of relative homogeneity in geographic characteristics expected to influence ecological conditions in lakes (Larsen and Christie 1993). Locations of some lakes are adjusted slightly for cartographic purposes.



assemblage surveys published during the 1920s and 1930s (e.g., NYSDC 1927–1939; NHFGC 1937–1939) have generally not been repeated. In addition, most regional surveys have selected lakes or rivers nonrandomly, such that the statistical representativeness of the data to the region as a whole is unknown.

In this paper, we assess regional-scale distributions and rates of occurrences of native minnows in lakes in the northeastern United States (New York, New Jersey, and the six New England states). We review the ichthyogeography of the Northeast and historical survey data. We discuss native ranges and propose regional species pools for lake minnows in the Northeast. We then analyze the relationships among factors affecting current minnow occurrences: geographic patterns, introduced and native littoral predators, and human disturbances along the shoreline and in the watershed.

Methods

Sampling design and field methods

The data for this paper were from the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) northeast lakes pilot, a synoptic survey designed to answer questions about the numbers, spatial extent, and ecological condition of surface water resources at a regional scale (Larsen et al. 1991, 1994; Larsen and Christie 1993; Whittier and Paulsen 1992; Stevens 1994).

We sampled fish assemblages in 179 lakes and reservoirs during the summers of 1992–1994 in the northeastern United States (Fig. 1). These lakes were selected, using a systematic random design, from all lakes >1 ha. Selection probabilities differed among lake size-classes to compensate for the preponderance of small lakes, and geographic clustering was used to assure a spatially distributed sample (Larsen

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et al. 1991, 1994; Stevens 1994). Lakes were excluded from sampling only if they were very shallow (<1 m), had <0.1 ha open water, were >10 000 ha, or access permission was denied by landowners. This probability sample represents a target population of an estimated 11 080 lakes in the Northeast (Whittier et al. 1995). A subset of 37 lakes were sampled up to four times over two summers. Unless otherwise noted, only one visit's data were used in this study to maintain uniformity in sampling effort. We also used data from 16 additional New England lakes, purposively selected and sampled in 1991 for methods evaluation (Paulsen et al. 1991; Larsen and Christie 1993).

Fish assemblages were sampled with overnight sets of gillnets, trapnets and minnow traps, and by night seining (Whittier et al. 1997). The level of effort was determined by lake size, ranging from 1 to 10 sets of each passive gear and up to 6 seining sites. Monofilament experimental gillnets (40 × 1.5 m) contained 14 randomly ordered panels of 6.25 mm to 60 mm bar mesh and were bottom set in the hypolimnion, metalimnion, and epilimnion and suspended in the pelagic epilimnion. Oneida-style trapnets (2 × 1 m frames) were deployed in each major littoral habitat, with minnow traps placed nearby. Two 25 m hauls were done with an 8.7 × 1.2 m beach seine (5 mm mesh) at each seining site. If adequate suitable habitat was not available for beach seine use, seining was supplemented with a 2.7 × 1.1 m short seine (5 mm mesh). For this study, we combined data from all gear.

The sampling objective was to collect a representative sample of the fish assemblage at each lake. Fish species were not targeted nor were habitats judged as fish rich (e.g., inlets, shoals). Sites were selected using a stratified random design, except for seining because beaches are rare in northeastern lakes. Littoral fish sampling was done at random stations within each macrohabitat class. Pelagic sample sites were chosen in random directions from the deepest location. The collected fish were identified to species and counted. Vouchers were examined to verify identifications and archived at the Harvard University Museum of Comparative Zoology Cambridge, Mass., or the New York Biological Survey Museum, Troy.

Littoral and riparian habitat was assessed at 10 preselected stations evenly spaced along the shoreline. At each station, crews recorded the presence of any of 11 kinds of human influence visible within a 15 m × 15 m riparian plot and the adjacent 10 m × 15 m littoral plot (Kaufmann and Whittier 1997) They also recorded the presence and estimated coverage of a variety of physical habitat features (e.g., aquatic and riparian vegetation). Field methods are detailed in Baker et al. (1997), which is available from either author at the Corvallis address. Field and laboratory data from this study may be found on the EMAP's website (http://www.epa.gov and search on EMAP) or may be obtained from the senior author.

Zoogeographic and landscape analyses

To determine native ranges for minnows and littoral predators, we examined species maps and descriptions in a variety of texts (Kendall 1914; Hubbs and Cooper 1936; Hubbs and Lagler 1964; Scott and Crossman 1973; Lee et al. 1980; Trautman 1981; Becker 1983; Smith 1985; Schmidt 1986; Underhill 1986; Page and Burr 1991), and state biological survey reports from New York (NYSDC 1927–1939) and New Hampshire (NHFGC 1937–1939). We used these sources in combination with our collections to determine which minnow species should be expected to occur in lakes, as opposed to primarily inhabiting streams or rivers. We synthesized this information into a series of sketched native range maps, from which we developed a map of lake minnow richness regions for the Northeast; broad areas that share the same pool of native lake minnows.

Current species distributions and frequency of occurrence in lakes were determined by mapping our collections. We examined geographic patterns in minnow and predator species presence and richness in the sampled lakes. We compared our collection data with the above range maps to judge whether a species was native for each lake. We then looked for areas of possible changes in individual or total species richness by comparing our data with the species pool map and accounts in the state biological surveys. We also compared all maps with a variety of broad-scale geographic features, such as ecoregions, human population densities, land use, and climate.

For each lake, we estimated the extent and intensity of human disturbance as the number of physical habitat stations with any of 11 types of visible human influence in the littoral (e.g., docks, fill) or immediate riparian areas (e.g., lawns, roads), and the average number of types of disturbance per station (P. Kaufmann, unpublished data). To estimate human disturbance at the watershed scale, we used digitized watersheds overlaid with coverages of human population density and road density (Census Bureau 1991; USEPA 1992), and wastewater discharge data (USEPA 1987) and land use – land cover (USGS 1990) from which proportions of the watershed in urban, industrial, commercial, residential, forested, wetlands, and agricultural categories were calculated.

The Northeast was divided into three subregions of relative homogeneity in landscape types and stressors judged likely to influence the ecological condition of lakes (Larsen and Christie 1993). Overlay maps of elevation, soil type, surficial and bedrock geology, potential natural vegetation, slope, climate, physiography, population density, land use – land cover, and ecoregions were examined for concordance of geographic patterns (C. Burch Johnson, unpublished data). The Adirondack Mountains (ADIR) was a distinct, lake-rich subregion. The remaining areas were grouped into lowland and upland subregions. The Coastal Plain, Great Lakes Plains, Hudson Valley, and Mohawk Valley were combined with the Allegheny Plateau into the lowland subregion (CLP). The upland subregion (NE-UP) consisted of the New England Mountains and New England Uplands (Fig. 1). We used these subregions as classes to aid evaluation of natural and human induced patterns.

Statistical approach

We used biplots, regressions, and chi-square analyses to examine relationships among minnow and predator species richness and possible controlling factors such as lake size, shoreline disturbance, and watershed disturbance. We regressed the number of minnow species and predator species against these indices. To partition the variability in the data, we ran the regressions using subregion and number of predator species as classes. To develop a watershed-scale estimator of human disturbance we ran a series of principal components analyses (PCA) (Gauch 1982; SAS Institute Inc.1985, PROC PRINCOMP) of the land cover and population variables. To help clarify interactions among these variables, we ran multiple regressions (SAS Institute Inc. 1985, PROC STEPWISE) for minnow and predator species richness, using lake size, shoreline and watershed disturbance, and predator richness as predictors.

Results

We collected 21 native minnow species on 263 visits to 195 lakes (Table 1, includes minnow scientific names). A map of minnow species richness (Fig. 1) shows a concentration of lakes with high numbers of minnow species in northern Maine where most lakes had three to seven species comprising 40–80% of species caught in each lake. Conversely, lakes in New Jersey, southern New York, and southern New England rarely had any minnows, other than golden shiner. Numbers of lake minnows were intermediate in the Adirondacks and the remainder of New England.

Minnow native ranges

Based on our literature review, all 21 minnow species collected inhabit streams while 15 also regularly occur in lakes (Table 1). (For simplicity, we refer to these species as "lake

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Table 1. Minnow species and numbers of lakes where collected in the northeastern United States by EMAP, 1991–1994 (n = 195).

Species	EMAP lakes	Native distribution in northeastern United States
Lake dwellers		
Golden shiner (*Notemigonus crysoleucas*)	134 (69%)	Entire region (except northern Maine and Adirondacks)
Creek chub (*Semotilus atromaculatus*)	40 (21%)	Entire region
Fallfish (*Semotilus corporalis*)	38 (19%)	Entire region (except Adirondacks)
Common shiner (*Luxilus cornutus*)	35 (18%)	Entire region
Lake chub (*Couesius plumbeus*)	18 (9%)	Northern (and stream populations in northwestern Massachusetts)
Northern redbelly dace (*Phoxinus eos*)	18 (9%)	Northern (and stream populations in northwestern Massachusetts)
Pearl dace (*Margariscus margarita*)	17 (9%)	Northern (and stream populations in western New York)
Bluntnose minnow (*Pimephales notatus*)	12 (6%)	Western (except Adirondacks)
Finescale dace (*Phoxinus neogaeus*)	10 (5%)	Northern
Bridle shiner (*Notropis bifrenatus*)	7 (4%)	Lowlands – entire region (except northern and eastern Maine and western New York)
Blacknose shiner (*Notropis heterolepis*)	7 (4%)	Northern
Spottail shiner[a] (*Notropis hudsonius*)	7 (4%)	Western (except Adirondacks), possibly Connecticut River drainage
Fathead minnow (*Pimephales promelas*)	6 (3%)	Northern Maine & Western
Blackchin shiner (*Notropis heterodon*)	3 (2%)	Western
Mimic shiner (*Notropis volucellus*)	3 (2%)	Western (except Adirondacks)
Phoxinus intergrades[b]	3 (2%)	Northern
Stream and river dwellers		
Blacknose dace (*Rhinichythys atratulus*)	10 (5%)	Entire region
Spotfin shiner (*Cyprinella spiloptera*)	3 (2%)	Western (except Adirondacks)
Eastern silvery minnow (*Hybognathus regius*)	3 (2%)	Western (except Adirondacks), possibly Connecticut River drainage
Cutlips minnow (*Exoglossum maxillingua*)	2 (1%)	Western, possibly Housatonic River drainage
Rosyface shiner (*Notropis rubellus*)	1 (–)	Western
Longnose dace (*Rhinichthys cataractae*)	1 (–)	Entire region

Note: These data include repeat visits within or among years. Numbers in parentheses are the frequency of occurrence (percentage of sampled lakes). Lake dweller is a species expected to reside in lakes, as well as streams. Stream and river dwellers are species rarely residing in lakes. Western = west and inclusive of Hudson River and Lake Champlain drainages.

[a]Spottail shiner appears to be limited to large water bodies.

[b]At three lakes the only *Phoxinus* collected were intergrades and were treated as a minnow species in this study. Hybrids were not counted as a species at any other lakes.

dwellers"; most are also widespread in streams and rivers.) Blacknose dace is usually not considered a lake dweller, but occurred in a higher proportion of EMAP lakes than some uncommon lake species. In much of the Northeast, it is the most commonly encountered stream minnow (NHFGC 1937–1939; Halliwell 1989). Our life-history review of the 23 other native Northeast minnows indicated that no lake dwellers were missed by our sampling, except for brassy minnow (*Hybognathus hankinsoni*). The NYSDC (1927–1939) reported it as sometimes abundant and fairly widespread in lakes and ponds in the northern Adirondacks.

Four lake species are broadly distributed throughout the Northeast: golden shiner, creek chub, fallfish, and common shiner. The golden shiner is nearly ubiquitous in Northeast lakes occurring in 69% of the EMAP lakes (Fig. 2), making it the third most widespread lake species after pumpkinseed (*Lepomis gibbosus*) and yellow perch (*Perca flavescens*) (T. Whittier, unpublished data). It is a popular baitfish introduced into many lakes and is probably not native to upland Adirondack lakes (NYSDC 1927–1939) and northern Maine (Kendall 1914). Because of its ubiquity, generally we will exclude the golden shiner from further analyses or discussion. The common shiner was usually listed as a stream species, but it inhabits lakes in the northern portion of its range (Scott and Crossman 1973; Page and Burr 1991). Our data supported this (Fig. 2).

There are five northern species (Fig. 3): lake chub, northern redbelly dace, finescale dace, pearl dace, and blacknose shiner, generally limited to northern New England, northern New York, and near the Great Lakes. Minor exceptions are: (*i*) limited disjunct stream populations of lake chub and northern redbelly dace in northwestern Massachusetts (Halliwell 1989) and (*ii*) an extensive stream population of pearl dace in southwestern New York (Smith 1985).

Three species, bridle shiner, fathead minnow, and bluntnose minnow, are lake-dwellers that were collected in relatively few of the lakes (Fig. 4) but that have fairly wide native distributions. Bridle shiner is native to lowland areas from southwestern Maine to central New York. The original distribution of fathead minnow is uncertain but probably is west of the Champlain and Hudson divides and in northern Maine (Kendall 1914). The bluntnose minnow is native west of the Champlain and Hudson divides, but not to the Adirondacks (NYSDC 1927–1939). The latter two are commonly used for bait and have been widely introduced outside of their original ranges.

Mimic shiner and blackchin shiner are also expected in lakes, but are at the edge of their ranges in this region. The mimic shiner is a western species found in western New York and in Lake Champlain. The blackchin shiner's range is more northwesterly. It is a species of concern in New York, and apparently declining (Smith 1985). The spottail shiner, a western species which prefers large waterbodies, was collected in larger lakes (>100 ha), mostly reservoirs. Three other western

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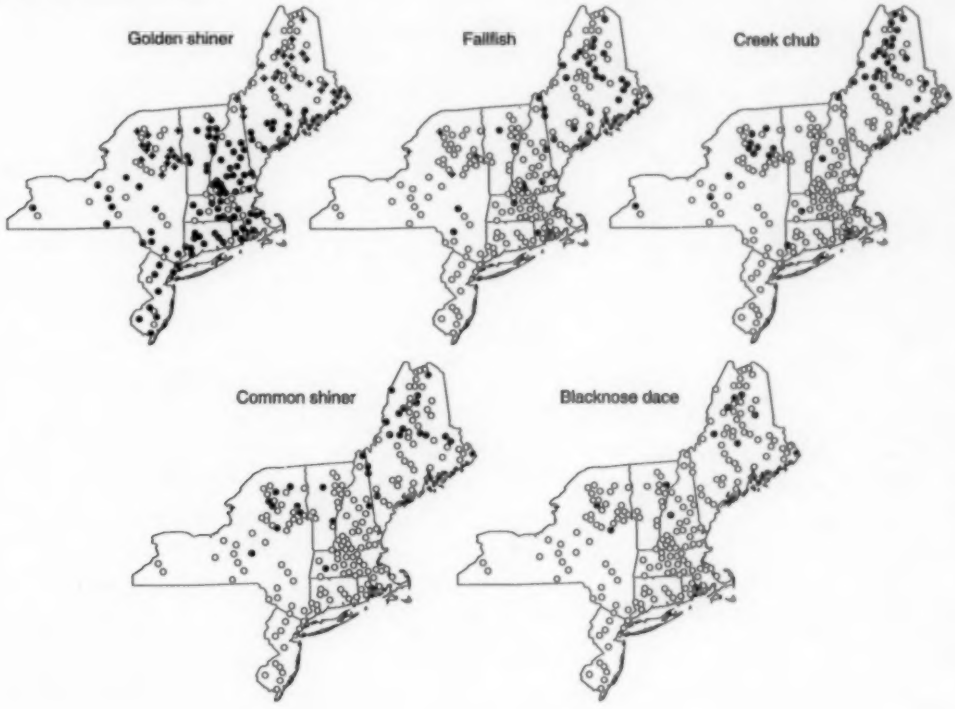
Fig. 2. Five minnow species are generally native to lakes and streams throughout the entire northeastern United States. Golden shiner is probably not native to northern Maine and the Adirondacks. Fallfish is probably not native to the Adirondacks. The solid symbols indicate lakes where these minnows were collected at least once by EMAP during summers 1991–1994. A plus sign indicates collections in lakes where the species are probably not native.



stream minnows occasionally collected were: cutlips minnow, eastern silvery minnow, and spotfin shiner. Two stream species, longnose dace and rosyface shiner were collected in only one lake each.

Regional species pools

We synthesized the species range information to produce a map of native lake minnow species richness (Fig. 5), showing the pool of potential species. The clearest differences are between north and south; two to three times as many lake dwelling minnows occur in the northern areas. We drew the north–south divide as a broad transitional zone, with a fairly sharp drop in numbers of native lake minnow species across this zone. In New England, this north–south divide generally follows the boundary between the Northeast Highlands and Northeast Coastal Lowlands ecoregions (Omernik 1987) but, in New York, does not consistently coincide with any commonly used regional framework (e.g., ecoregions (Omernik 1987), physiographic regions (Fennemen and Johnson 1946)).

The second pattern in species richness is an increase in minnow species to the west, in particular across the Champlain and Hudson divides. The native fish fauna in New England is fairly depauperate due to limited invasion opportunities since the last ice age (Schmidt 1986; Halliwell 1989). All 17 native New England minnows were collected in EMAP lakes, at least occasionally (Table 1). There are more than twice as many native minnow species ($n = 43$) west of the Hudson and Champlain divides (D. Halliwell, unpublished data), but only three of the additional species can be considered to be lake dwellers.

Finally, it appears that there are no native lake minnows in the New Jersey coastal plain, except golden shiner and possibly spottail shiner. We collected no native minnows, except golden shiner in this area. All maps we reviewed showed ranges of other lake dwelling species ending at the edge of the piedmont in New Jersey. However, using presence data from 41 New Jersey coastal plain lakes collected in 1950–1952, Graham (1993) reported ironcolor shiner (*Notemigonus chalybaeus*) in four lakes, eastern silvery minnow in two lakes, common shiner in one lake, and spottail shiner in three lakes. The first three are stream dwellers. Spottail shiner is usually restricted to large water bodies. Thus, we excluded our seven relatively small lakes in this area from further analysis.

Littoral predators

The data showed an inverse relationship between occurrences of minnows and certain predator types. The clearest pattern was with species that occupy the littoral zone all year (i.e., share minnow habitat) and are highly piscivorous, eating primarily fish beginning in their first year. We selected five littoral top predators as typical of those characteristics: northern pike (*Esox lucius*), chain pickerel (*E. niger*), largemouth (*Micropterus salmoides*) and smallmouth (*M. dolomieu*) basses, and white perch (*Morone americana*). Chain pickerel is the only littoral predator native to most of the Northeast,

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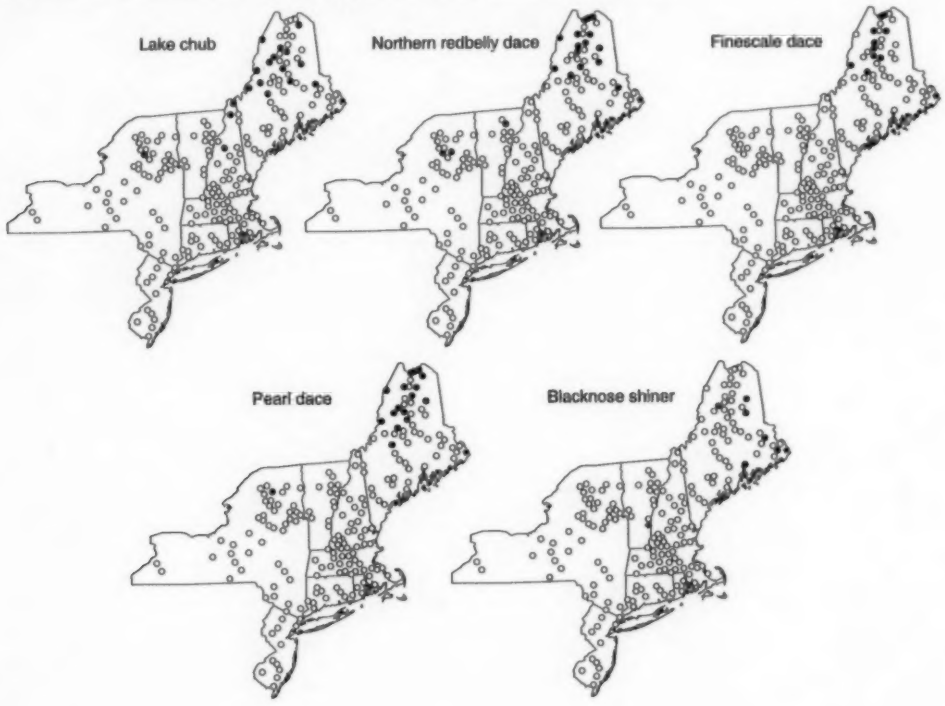
Fig. 3. Five lake-dwelling minnow species have native ranges limited to the northern portions of the Northeast. The solid symbols indicate the lakes where these minnows were collected at least once by EMAP during summers 1991–1994.



except upland Adirondacks and upland areas of northern New England (Fig. 6). The two black basses are native only to lowland areas of Great Lakes and St. Lawrence drainages in this region but have been widely introduced elsewhere. The northern pike's native range is similar to the black basses but also includes Lake Champlain and adjacent lowland areas. White perch originally only occurred in coastal lakes with low-gradient connections to the coast but have been introduced as sportfish into numerous inland waters.

The characteristic of piscivory is clearly a continuum; numerous species occasionally take other fish. Species not meeting the above characteristics appear to have little effect on northeastern lake minnows. Burbot (*Lota lota*) does not usually use the littoral zone in the summer. We collected burbot in nine northern Maine lakes that were among the most minnow rich lakes in our data (mean 5.4 species). Salmonids tend not to be littoral in the summer and not piscivorous in the first year(s). In northeastern lakes the most piscivorous salmonids are landlocked Atlantic salmon (*Salmo salar*) and lake trout (*Salvelinus namaycush*). In lakes with either species, minnow richness was relatively high unless one or more of the littoral predators (above) were also present. Brook trout (*Salvelinus fontinalis*) is the most widespread salmonid in the Northeast and typically coexists with a rich minnow assemblage.

Of the 188 lakes within the native ranges of lake-dwelling cyprinids, 47% had minnows other than golden shiner, 73% had littoral predators, and 7% had neither (Table 2). Of the lakes with minnows, 60% also had predators; of those lacking minnows, 86% had predators. Lakes with predators tended not to have native minnows ($\chi^2 = 7.42$, $p = 0.006$) and lakes lacking predators tended to have minnows ($\chi^2 = 9.6$, $p = 0.002$). The median number of minnow species in lakes without predators was two, while it was zero in lakes with predators.

On a regional scale, introduction of non-native predators has changed the fish assemblage structure in northeastern lakes. In 35% of lakes, all littoral predators were non-native (Table 2). Non-native predators were present in 69% of lakes. Native predators were the only predators in 5% of lakes and present in 38%. A comparison of the proportions of lakes with and without minnows to lakes with native and non-native predators (Tables 2 and 3) indicates that introduced predators were no more effective at eliminating minnows from lakes than are the native predators, or that lake minnows in the Northeast coexist no better with native littoral predators than with non-natives.

If introduced predators were no more likely than natives to eliminate minnows from lakes, were they more likely to make minnows "rare"? We designated a minnow species as rare at a lake if fewer than five individuals were collected. In 19 lakes, all minnow species were rare in our collections (Tables 3 and 4). The column percentages in Table 3 show no evidence that introduced predators had a greater effect than natives. Minnows were less likely to be rare in lakes with no predators ($\chi^2 = 6.41$, $p = 0.011$). Littoral predators in general seemed to suppress abundances of minnows.

We examined the effect of increasing predator species richness on minnow presence and rarity. There was a significant but weak relationship between lake size and number of minnow species, as well as predator species (Table 5). When lakes

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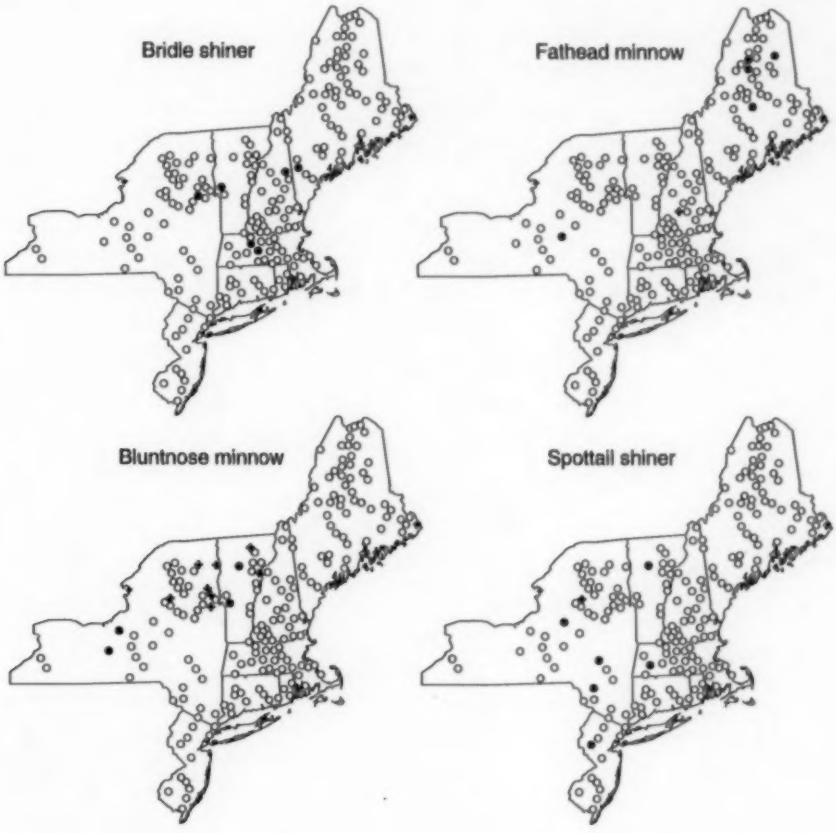
Fig. 4. Four other minnow species were collected in more than six lakes by EMAP during 1991–1994. The bluntnose minnow is probably not native to the Adirondacks and eastern Vermont. The fathead minnow is probably not native to New Hampshire. The spottail shiner may have been introduced to the Connecticut River system and the Adirondacks early this century. The solid symbols indicate lakes where these minnows were collected at least once by EMAP during summers 1991–1994. A plus sign indicates collections in lakes where the species are probably not native.



were classified by the number of predator species, each "additional" predator species reduced the number of minnow species (i.e., reduced the regression slope) (Tables 4 and 5, Fig. 7). The relationship between lake size and minnow species richness was stronger for each class 0–2 predator species than for the unclassified data (Table 5). The data in Table 4 does not show that increasing numbers of predator species (more than one) pushes minnows toward local rarity. However, in half of the lakes where minnows coexisted with three or more predator species, fallfish was the only cyprinid collected (other than golden shiner).

Finally, we evaluated which minnow species and groups appeared to be tolerant or intolerant of littoral predators. Other than golden shiner, fallfish was most likely to coexist with predators in lakes; it was collected 29 times in lakes with predators (11 times as a rare species) but only 8 times in lakes lacking predators. Fallfish made up 15% of all minnow collections, 30% of collections in lakes with two or more predators, and 25% in lakes with any predators. Fallfish is the largest native minnow in the Northeast (adult total length 100–240 mm), which undoubtedly aids in avoiding predation.

Seven other minnows appear to be tolerant of littoral predators. Common shiner was collected in 15 lakes lacking predators and 20 lakes with predators (seven times as a rare species), comprising 17% of minnow collections in lakes with predators. The bridle shiner was collected once in lakes without predators and in five lakes with predators (three times as rare). The bridle shiner is a lowland, warmwater species and may be somewhat adapted to littoral predators. The other five possibly predator-tolerant species all have western ranges: blackchin shiner, bluntnose minnow, mimic shiner, spotfin shiner, and spottail shiner. Clearly the number of collections for most of these are too low (Table 1) to adequately evaluate their tolerance to predators.

Creek chub and blacknose shiner appeared to be moderately tolerant of predators. The other six species rarely occurred with predators. Four of these are northern minnows. The five northern species comprise 46% of the minnow collections in lakes lacking predators but only 11% of minnow collections in lakes with predators.

Habitat and watershed disturbance

A plot of the number of minnow species against increasing shoreline disturbance (Fig. 8) showed the wedge-shaped pattern

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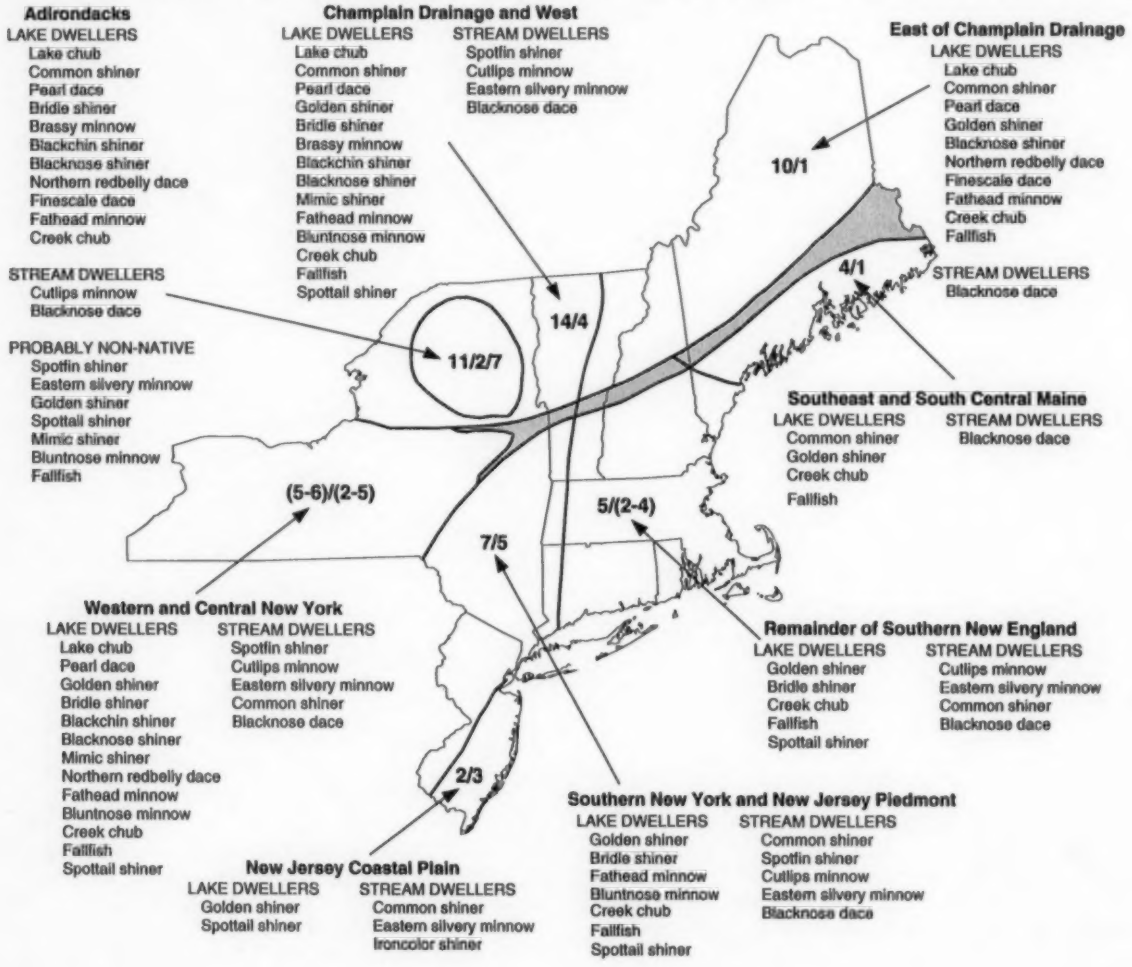
Fig. 5. Species pools of lake dwelling minnows. For each area the first number is the number of lake dwelling minnow species native to that area. The second number is the number of species considered as primarily stream dwelling occasionally collected in lakes in that area. In the Adirondacks, the third number represents non-native minnows collected in lakes. In western New York, the pattern of native ranges is complex; the total number of potential lake dwelling species is 13 plus 5 stream dwelling species, but in any particular location the maximum is 5 or 6 lake dwellers plus 2–5 stream dwellers. In southern New England, the number of stream dwellers increases from two in the east to four in the west. The golden shiner is native in all areas except northern New England and the Adirondacks. Spottail shiner appears to be restricted to large water bodies. We treat common shiner as a lake dweller in the north and primarily as stream dwelling to the south and west.



of points typical of species–habitat data, where the particular habitat variable was one limiting factor (Terrell et al. 1996; P.R. Kaufmann, unpublished data). Ordinary least-square regressions of these kinds of data are not very useful for detecting interesting patterns (Terrell et al. 1996), as was the case here (Table 5). However, visual examination of the plot indicated that increasing human shoreline activity set a decreasing upper bound on the number of minnow species present. Only two lakes with more than two disturbed stations had more than three species, while only three lakes with more than seven disturbed stations had any minnows.

Subsetting these data by the number of predator species revealed two additional patterns (Fig. 8). First, increasing predator species richness moderated the effect shoreline disturbance had in limiting minnow species richness; the habitat effect disappeared when predator species exceeded one. Second, the lakes with more than 50% disturbed shoreline stations always had predators. Of the lakes with ≥50% disturbed shoreline, 68% had two or more predator species. This does not imply that littoral predators thrive in disturbed conditions. Rather, it seems that, as permanent human activity at a lake increases, so does the likelihood that humans will introduce predators.

We ran several PCAs of watershed-level human disturbance variables, which all produced first PC axes that accounted for >60% of the variance and described the same general picture of watershed-scale disturbance. Several of the variables were highly correlated; thus, we reduced the variable

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Fig. 6. Five littoral predator species were collected in Northeast lakes. Solid symbols indicate the locations where these species were collected at least once by EMAP during summers 1991–1994. A plus sign indicates collections in lakes where the species is probably not native.

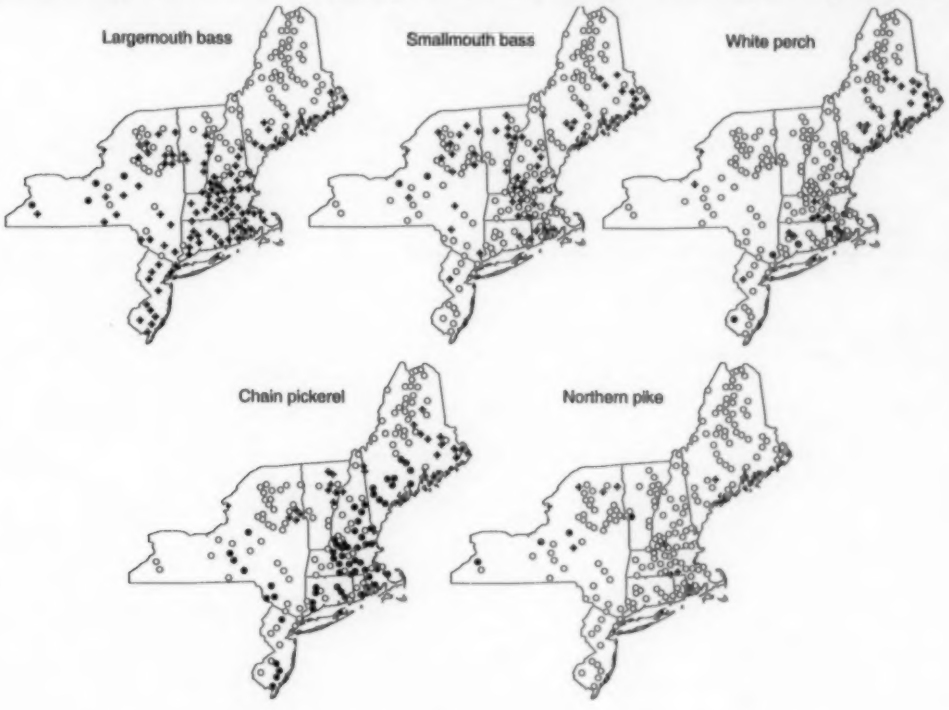

Table 2. Proportions of lakes sampled by EMAP 1991–1994 in the Northeast with littoral predators and minnows.

	All lakes (n = 188)	Lakes with predators (n = 134)	Lakes without predators (n = 54)	Lakes with minnows (n = 93)	Lakes without minnows (n = 95)
Predators (%)	73.4	100	na	59.6	85.9
All predators are non-native (%)	35.1	47.8	na	37.1	33.3
All predators are native (%)	4.8	6.5	na	4.5	5.0
Both native and non-native (%)	33.5	45.6	na	18.0	47.5
Minnows (%)	47.3	38.4	72.0	100	na
No predators or minnows (%)	7.4	na	na	na	na

Note: Data do not include golden shiner or lakes in the New Jersey coastal plain. Littoral predators are largemouth bass, smallmouth bass, chain pickerel, northern pike, and white perch. na = not applicable.

set by keeping only one of each highly correlated set. The first principal component (PC1), using five variables (road density, percent agricultural land, percent forest, percent urban land, and population density) accounted for 62% of the variance, with all variables loading fairly evenly (Table 6). Human land uses loaded positively and forests loaded negatively on PC1. The second principal component (PC2) accounted for 17.5% of the variance and was mainly an agriculture axis.

The relationship between number of minnow species and PC1 scores for watershed disturbance (Fig. 9) was similar to that for minnows and shoreline disturbance (Fig. 8) and was generally constrained by littoral predators. Similarly, there was a significant but weak relationship (Table 5) between increasing number of predator species and increasing PC1 scores (greater human activity in the watershed).

For both minnow and predator species richness in Northeast lakes, forward stepwise regression (SAS Institute Inc. 1985)

Table 3. The number of lakes with native minnows (exclusive of golden shiner) either absent, rare (all minnow species with less than five individuals collected), or not rare, related to whether littoral predators are native, non-native, both, or absent.

	Littoral predator type				
	Non-natives only	Natives only	Both	Absent	Totals
Minnows absent	23 (50.0)	5 (55.68)	47 (74.6)	14 (28.0)	99 (52.7)
Minnows rare	10 (15.13)	2 (22.25)	5 (7.9)	4 (8.0)	21 (11.2)
Minnows not rare	23 (34.8)	2 (22.2)	11 (17.5)	32 (64.0)	68 (36.2)
Totals	66	9	63	50	188

Note: Values given in parentheses are the column percentages.

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Table 4. The number of lakes with native minnows (exclusive of golden shiner) either absent, rare (all minnow species with less than five individuals collected), or not rare, related to the number of littoral predator species.

	Number of littoral predator species						
	0	1	2	3	4	5	Totals
Minnows absent	14 (28.0)	24 (53.3)	40 (72.7)	13 (54.2)	7 (53.8)	1 (100)	99
Minnows rare	4 (8.0)	6 (13.3)	8 (14.6)	2 (8.3)	1 (7.7)	0	21
Minnows not rare	32 (64.0)	15 (33.3)	7 (12.7)	9 (37.5)	5 (38.5)	0	68
Total lakes	50	45	55	24	13	1	188
Minnow collections	126	46	27	17	8	0	224
Mean minnow species (lakes with minnows)	3.6	2.2	1.8	1.5	1.3		
Median minnow species (all lakes)	2	0	0	0	0	0	

Note: Values given in parentheses are column percentages.

Table 5. Selected regression models for the number of minnow and littoral predator species.

Predictor(s) and data set		Model	p	r^2	N
		Minnows			
Lake size	All lakes	0.84(AREA) – 0.17	0.0001	0.16	188
	Predators = 0	1.91(AREA) – 0.32	0.0001	0.48	54
	Predators = 1	1.25(AREA) – 0.73	0.0001	0.49	43
	Predators = 2	0.77(AREA) – 0.72	0.0001	0.29	53
	Predators >3	0.44(AREA) – 0.30	0.056	0.10	38
	CLP	0.37(AREA) – 0.15	0.001	0.16	64
	ADIR	0.83(AREA) – 0.12	0.02	0.22	24
	NE-UP	1.11(AREA) – 0.17	0.0001	0.19	100
Shoreline disturbance	All lakes[a]	–0.16(SHORE) + 1.63	0.0003	0.08	171
Watershed disturbance	All lakes[b]	–0.42(WTRSHD) + 1.23	0.0001	0.19	162
	NE-UP[b]	–0.72(WTRSHD) + 1.28	0.0001	0.24	82
Lake size and predators	All lakes[c]	1.28(AREA) – 0.79(PRED) + 0.30	0.0001	0.46	162
	NE-UP[c]	1.34(AREA) – 0.97(PRED) + 0.66	0.0001	0.50	82
Lake size, predators, and watershed disturbance	All lakes[c]	1.18(AREA) – 0.68(PRED) – 0.15(WTRSHD) + 0.30		0.48	162
	NE-UP[c]	1.21(AREA) – 0.82(PRED) – 0.27(WTRSHD) + 0.50	0.0001	0.54	82
		Predators			
Lake size	All lakes	0.58(AREA) + 0.52	0.0001	0.14	188
	CLP	1.06(AREA) + 0.45	0.0001	0.55	64
	ADIR	0.68(AREA) – 0.22	0.005	0.31	24
	NE-UP	0.29(AREA) + 0.72	0.06	0.04	100
Shoreline disturbance	All lakes[a]	0.15(SHORE) + 1.09	0.0001	0.13	172
	ADIR[a]	0.24(SHORE) + 0.36	0.0001	0.51	24
	NE-UP[a]	0.18(SHORE) + 0.89	0.0001	0.16	88
Watershed disturbance	All lakes[b]	0.29(WTRSHD) + 1.48	0.0001	0.17	162
	ADIR[b]	0.81(WTRSHD) + 1.99	0.0002	0.50	22
	NE-UP[b]	0.41(WTRSHD) + 1.50	0.0001	0.22	83
Lake size and shoreline disturbance	CLP[a]	1.039AREA) + 0.07(SHORE) + 0.23	0.0001	0.56	57
Lake size, watershed & shoreline disturbance	All lakes[c]	0.63(AREA) + 0.25(WTRSHD) + 0.09(SHORE) + 0.21	0.0001	0.37	162
	ADIR[c]	0.30(AREA) + 0.41(WTRSHD) + 0.11(SHORE) + 0.68	0.0004	0.62	22
	NE-UP[c]	0.39(AREA) + 0.32(WTRSHD) + 0.11(SHORE) + 0.57	0.0001	0.32	83

Note: Nonsignificant models and those with very low correlations are not included. AREA = $\log_{10}$(lake surface area (ha)); SHORE = number of shoreline stations with overt human activity; WTRSHD = PCA axis 1 scores of five measures of human activity in the watershed (higher values imply greater human impact in the watershed; see Table 6); PRED = number of littoral predator species.

[a]Shoreline disturbance data were not available for 16 lakes sampled in 1991.

[b]Watershed disturbance data were not available for 16 lakes sampled in 1991 and 10 lakes sampled in 1992.

[c]Only lakes with watershed disturbance data available were used.

chose watershed disturbance first and lake size second (Table 5), with increasing lake size positively related to richness for both, and increasing watershed disturbance negative for minnows but positive for predators. The stepwise procedure chose predator richness as a third predictor for minnow richness. However, a model using only lake size and predator richness as predictors for minnow richness achieved nearly the same correlation as the three-variable model (Table 5). The stepwise procedure added shoreline disturbance to the predator richness model (partial $r^2 = 0.03$).

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Fig. 7. Minnow species richness (excluding golden shiner) tended to increase with increasing lake size. There was a significant decrease ($p < 0.001$) in the expected number of native minnow species (adjusted for lake size) with increasing number of predator species as follows: minnow species$_{(pred = 0)}$ > minnow species$_{(pred = 1)}$ > minnow species$_{(pred = 2)}$. The decrease from two predators to more than three predator was not significant ($p = 0.35$). Regression models are given in Table 5.

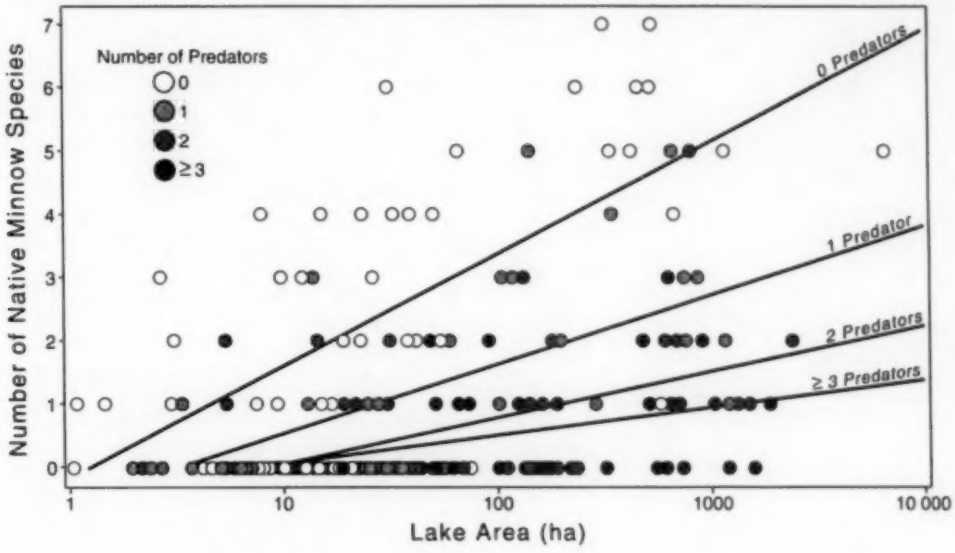

Fig. 8. Maximum minnow species richness decreased as the number of shoreline stations with any overt human activity increased. This pattern disappeared as the number of predators species increased.

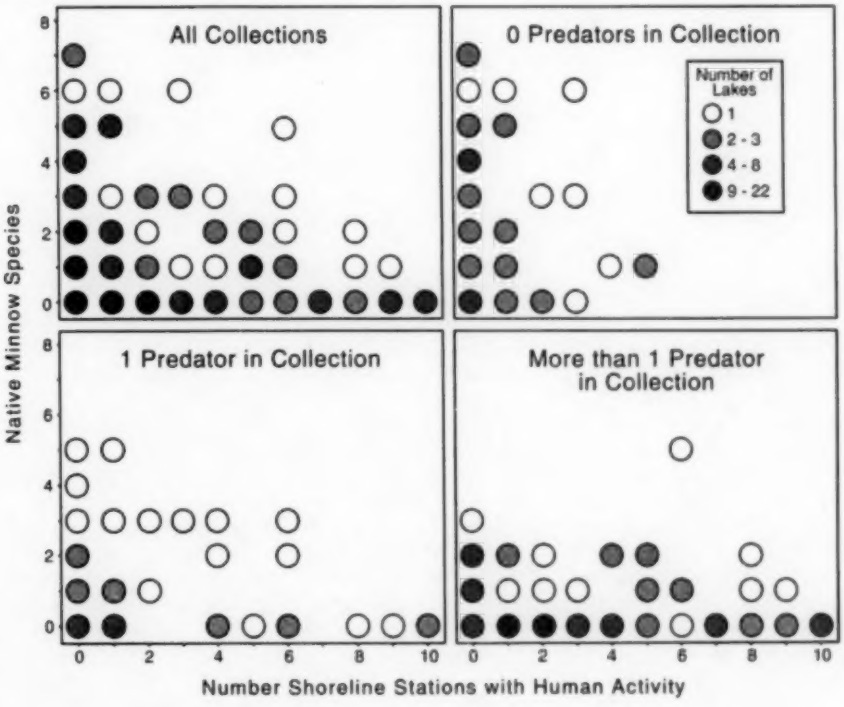

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Table 6. Principal components analysis of watershed-level human influence measures: three land use – land cover variables (% forest, % urban, % agricultural), human population density (no./km^2), and road density (km/ha).

(A) Principal components.

Principal component	Eigenvalue	Proportion of variance	Cumulative
PC1	3.09	0.617	0.617
PC2	0.88	0.175	0.792
PC3	0.58	0.116	0.909

(B) Eigenvectors.

	PC1	PC2	PC3
% Forests	–0.42	0.34	0.69
% Urban	0.49	0.31	–0.29
% Agricultural	0.32	–0.81	0.41
Population density	0.52	0.21	0.15
Road density	0.46	0.30	0.50

Note: All variables except % forest were $\log_{10}(x + 1)$ transformed. The first principal component (PC1) scores estimate generalized human influence. The second principal component (PC2) is an agriculture axis.

Subregional patterns

There were also subregional differences in the factors discussed above (Table 5). In the Coastal Lowlands Plateau subregion (CLP), only 17 of 86 (20%) lakes had any minnow species, with one lake having three species; all but 4 lakes had littoral predators. In the CLP, predator richness was fairly strongly correlated with lake size ($r^2 = 0.55$), while minnow richness was not ($r^2 = 0.15$); the relationships of minnow and predator richness to both human disturbance measures were not significant at the 0.1 level.

The Adirondacks (ADIR) had a greater proportion of lakes with minnows, greater minnow richness (maximum 5) and more lakes without littoral predators (11 of 24) than the CLP. In the ADIR there were significant but weak relationships between both minnow and predator richness and lake size (r^2 = 0.18 and 0.28, respectively; Table 5). Minnow richness was not significantly related to either shoreline or watershed disturbance in the ADIR, but predator richness was ($r^2 = 0.49$ and 0.47).

The New England Uplands (NE-UP) had the greatest minnow species richness (maximum seven). The minnow richness and lake size relationship ($r^2 = 0.18$; Table 5) was similar to the ADIR; there was no predator richness and lake size relationship. Unlike in the other two subregions, minnow richness was significantly and negatively related to shoreline and watershed disturbance ($p = 0.0001$ and 0.003, respectively) in the NE-UP, although correlations were still low ($r^2 = 0.08$ and 0.24, respectively). Predator richness showed a significant and positive but weak relationship with shoreline and watershed disturbance ($r^2 = 0.15$ and 0.21, respectively).

The stepwise regression chose only lake size as a predictor for minnow richness in both the CLP and ADIR. Adding predator richness to the model increased the r^2 to 0.27 in the ADIR but had no effect in the CLP. In the NE-UP the stepwise procedure selected predator richness first and lake size second (partial $r^2 = 0.27$ and 0.25 respectively); adding watershed disturbance increased the r^2 only by 0.02 (Table 5). Subregional multiple regression models for predator richness were more varied. In the CLP the stepwise procedure selected lake size first ($r^2 = 0.53$) and shoreline disturbance (partial $r^2 = 0.03$). In the ADIR, it selected watershed disturbance first ($r^2 = 0.50$), followed by shoreline disturbance and lake size (partial r^2 = 0.07 and 0.06, respectively). In the NE-UP the selection order was similar, but the total correlation was weaker ($r^2 = 0.32$). Note that the coefficients for shoreline and watershed disturbance are positive for all predator models, and negative for minnow models.

Other abiotic factors

Low pH and lack of connections to other water bodies also are likely to reduce minnow species richness (Tonn and Magnuson 1982; Rahel 1986; Robinson and Tonn 1989; Baker et al. 1990, 1993). All 14 lakes with pH <6.0 had no minnows or only golden shiner (in 5 lakes). Two Adirondack lakes were apparently fishless, and one was maintained by stocking. Five low-pH lakes were in the New Jersey coastal plain and had one or two littoral predator species. The remaining six low-pH lakes all had two or three littoral predators.

Eight lakes were seepage lakes (no inlet, no outlet) and two were closed (inlets, but no outlet). All of these are small (<11 ha). There were no minnows collected at eight of these lakes; the other two had one minnow species each. Six of the 11 lakes had littoral predators. Although lake isolation probably influences minnow species richness, in the Northeast these types of lakes are uncommon; other factors such as littoral predators, reclamation history, and lake origin (e.g., as quarries) may be more important.

Discussion

A comparison of the regional species pools (Fig. 5) with the distribution of minnow species richness in individual lakes (Fig. 1) indicates that some of the present geographic pattern in richness should be expected based on native species distributions, in particular the greater numbers to the north. However, some current patterns are not concordant with the native ranges: (*i*) the large number of southern lakes with no minnows; (*ii*) richness in the Adirondacks, northern Vermont, and New Hampshire seems lower than expected, when compared with northern Maine; and (*iii*) the expected increase in species richness to the west does not occur.

In our study the most consistent factor related to native minnow species richness in northeastern lakes was the presence of littoral predators. Other studies have demonstrated similar relationships between predators and minnow assemblages in lakes. Tonn and Magnuson (1982) showed that cyprinids and mudminnows (*Umbri limi*) dominated northern Wisconsin lakes in which winterkill conditions eliminated esocids and centrarchids, but were largely absent in lakes with predators. In the 1930s, New York state biologists noted diverse minnow assemblages present in Adirondack lakes to which black basses (*Micropterus* spp.) had not been introduced, and a lack of minnows where they had (NYSDC 1927–1939). Lakes that have lost minnow species since then were more likely to have black basses or esocids present in the 1980s than those which still had minnows (Baker et al. 1993). In northern Wisconsin, He and Kitchell (1990) showed decreases in minnow biomass owing to increased emigration and predation, after introducing

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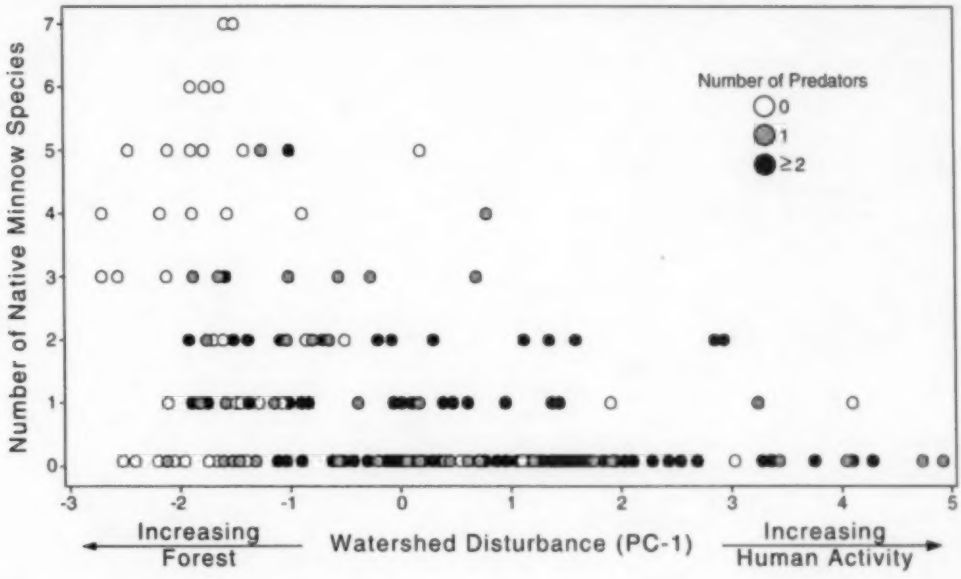
Fig. 9. Maximum minnow species richness decreased with increased human activity in the watershed. This pattern varied with the number of predator species. The horizontal axis is the first principal component of a PCA using % forest, % urban, % agriculture, road density, and human population density in each lake's watershed (Table 6) and is a generalized human impact score.



northern pike to a small lake previously lacking a top predator. Contrary to these examples, data contained in Pierce et al. (1994) showed no apparent relationship between numbers and species of littoral predators and minnow species richness in 10 southern Quebec lakes.

The Wisconsian glaciers left the Northeast rich in lakes but relatively impoverished of fish species, especially in New England (Schmidt 1986; Underhill 1986; Halliwell 1989). For most of the region, the chain pickerel is the only native littoral predator, generally restricted to lowland, warmwater habitats. The stocking programs of the last 100+ years have certainly changed the fish assemblage structure in Northeast lakes. Our data indicate that introduced predators per se have no greater effect on lake minnows than do the natives. The main effect comes from the addition of predator species, usually nonnative species.

In the absence of littoral predators, increasing human activity along the shoreline and in the watershed, was related to decreased minnow species richness. As humans settle around a lake we tend to alter the physical habitat by clearing the shoreline, removing woody debris (Christensen et al. 1996) and vegetation from the littoral zone, manipulating the hydrologic regime with water control structures, and increasing turbidity with motor boat activity. We also modify the chemical habitat, usually by increased nutrient loads but also with chemical treatment for weed and insect control, nonpoint-source runoff, and fisheries reclamation projects. These incremental impacts may eventually eliminate the native cyprinid assemblages. However, predators seem capable of doing the same thing, even where the habitat is relatively intact.

The interactions among minnows, human activity, predators, and geography are complex and intertwined to the extent that we cannot completely explain the current patterns in cyprinid distributions and species richness in northeastern lakes. Clearly, the greatest lake minnow richness occurs in some of the areas most remote from human populations in the eastern United States. Just as clearly, the native ranges of minnows and littoral predators lead us to expect greater minnow richness in those areas.

Minnow-rich, sparsely populated, northern Maine is an example of the results of these complex interactions. The climate and soils generally preclude agriculture but support extensive forests. Most of northern Maine is owned by a consortium of timber and paper companies, which limits access to the land, lakes, and most roads. The land is not generally available for development. Long driving times over unpaved roads from distant population centers also tends to discourage the ownership of second homes common in other lake-rich regions. Ironically, in this area, timber management appears to be beneficial for native fish assemblages. The climate and soils also produce lakes that support salmonids and lake-dwelling minnows. The local culture places great value on the salmonid sports fishery and generally opposes introduction of littoral predators (personal observations).

Compare northern Maine with the Adirondacks. Its climate, soils, and topography also favor forests over agriculture, and it has a low permanent population. At the watershed level, its land use is similar to that of northern Maine. However, Adirondack lakes are more likely to have overt human shoreline activity (e.g., cabins, boat docks, lawns, roads). Tourism and second homes, often associated with lakes, are major economic activities, along with logging. This area is closer to larger human population centers and has better and public roads. Although salmonids are highly valued as sportfish in the Adirondacks, so are basses and pike, which are not native. Finally, Adirondack lakes are more susceptible to acidification than lakes in northern Maine (Haines and Baker 1986).

Stocking of lowland game and forage species began in the

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Adirondacks before the first recorded sampling in the 1880s (Mather 1886, cited in NYSDC 1927–1939). Eight Adirondack lakes sampled by EMAP were discussed in NYSDC (1927–1939) with enough detail to qualitatively evaluate changes in cyprinid and predator assemblages. Four lakes had introduced littoral predators in the 1930s. Native minnows were listed as rare in these lakes; three had one non-native minnow. In the 1990s these four lakes still had non-native predators; five additional non-native minnow species were collected in one lake and two in another. Only one lake gained a native minnow. The other four lakes had no littoral predators in the 1930s and had either three or six native minnows, abundant or fairly common. In the 1990s, one of these lakes remained free of predators and had gained two native minnows. The other three lakes had gained one or three predator species and lost between one and five native minnows; two of these lakes also gained a non-native minnow, and one gained a native minnow.

The weight of evidence is that, except for northern Maine, human activity has reduced the β diversity of native cyprinids among Northeast lakes, especially in the Adirondacks. We do not have the historical data to quantify that loss, i.e., we cannot know whether minnows ever occurred in lakes in areas where they are now generally absent, such as southern New England or lowland New York. However, these data combined with the probability sample design provides a regional-scale snapshot of current species distributions and rates of occurrence in lakes.

If regional lake minnow diversity is declining, are any individual species at risk in the Northeast? At present, no lake minnow populations are listed as threatened or endangered by the states; only the blackchin shiner is a species of concern in New York. In Massachusetts, stream populations of lake chub and northern redbelly dace are listed as endangered, and eastern silvery minnow is of special concern.

Our data suggest that brassy minnow, blacknose shiner, blackchin shiner, and bridle shiner deserve additional attention in the Northeast. Sixty years ago, NYSDC (1927–1939) reported the brassy minnow to be widespread in lakes and ponds in the northern and eastern watersheds of the Adirondacks. EMAP collected none of this species. Currently, in New York, brassy minnows are rarely collected (R. Daniels, New York Biological Survey, Troy, N.Y., personal communication). Smith (1985) records one personal collection of blackchin shiner in New York, and we collected it twice in New York and once in Vermont. Anecdotal evidence and our data suggest that the blacknose shiner and bridle shiner are also becoming uncommon. Becker (1983) and Smith (1985) report that blacknose shiner are sensitive to habitat loss and water quality and are therefore declining. Smith (1985) shows 10 personal collection sites in New York for this minnow, and we collected it in seven New England lakes, with less than five individuals at three lakes. Of five major drainages in Virginia where the bridle shiner formerly occurred, it has been extirpated from one, and numbers are greatly reduced in two others (Angermeier 1995). In a 1993 survey designed to look for bridle shiner in small eastern Massachusetts lakes, they were found in only 9 of 36 lakes where historically collected, and in none of the 8 lakes previously lacking them (M. Chandler, New England Aquarium, Boston, Mass; unpublished data). We collected bridle shiner in seven lakes, with less than five individuals at three lakes.

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Influence of errors in natural mortality estimates in cohort analysis

G. Mertz and R.A. Myers

Abstract: The accuracy of the estimation of cohort strength from catch data may be greatly degraded if a poor estimate of the natural mortality rate is entered into the calculation. A straightforward, exact formulation for the error in cohort reconstruction due to a misspecified natural mortality rate is presented. The special case of constant fishing mortality is particularly transparent, allowing the error to be segmented into easily interpreted terms. A change in the fishing mortality may result in a distinct hump in the transient behavior of the bias factor, rather than a simple monotonic adjustment. This implies a similar pattern in estimated cohort strength.

Résumé : La précision de l'estimation de l'effectif d'une cohorte à partir des données de capture peut être réduite considérablement si on fait intervenir dans les calculs une estimation inexacte du taux de mortalité naturelle. On présente une formule exacte et simple pour corriger l'erreur dans la reconstruction d'une cohorte attribuable à un taux de mortalité naturelle mal précisé. Le cas spécial de la mortalité par pêche constante est particulièrement transparent, permettant la segmentation de l'erreur en termes facilement interprétables. Un changement dans la mortalité par pêche peut se traduire par une bosse distincte dans le comportement transitoire du facteur d'erreur, plutôt qu'en un simple ajustement monotone. Cela implique un changement semblable dans l'effectif estimé de la cohorte.
[Traduit par la Rédaction]

Introduction

The reconstruction of numbers-at-age from catch-at-age data is now a standard tool in fisheries science (Hilborn and Walters 1992). The techniques used for this reconstruction, e.g., cohort or virtual population analysis, are generically known as sequential population analysis (SPA). It is well appreciated that the result can be sensitive to the estimate of the natural mortality rate (M) assumed in the calculation. In this paper we provide a compact and illuminating expression for the error arising in SPA from the misspecification of M.

An appreciable number of investigations of errors in SPA precede this one: Pope (1972), Ulltang (1977), Sims (1984), Saila et al. (1985), Sampson (1988), Hilden (1988), Lapointe et al. (1989) and Aksland (1994). (This list is not intended to be exhaustive.) For our purposes we wish to highlight two results from these studies. First, implied by Sims (1984), Sampson (1988), and Aksland (1994), misspecification of M creates particularly large biases for lightly fished stocks. Second, changes in fishing mortality in the presence of a misspecified M, implied by Hilden (1988) and Lapointe et al. (1989), lead to a time-varying bias, thus imposing spurious time trends in SPA estimates of cohort size.

In regard to the first point, we show that it is possible to exactly formulate the error in cohort reconstruction, arising from misspecification of M, avoiding the Taylor series type approximations or simulations employed in earlier studies. The case in which a cohort is subjected to a constant fishing mortality proves to be particularly enlightening, in that one can clearly separate different error terms and interpret them.

In regard to the second point, we show that bias arising from misspecified M may, in the presence of a change in the fishing mortality, exhibit a lobe-shaped time trend, rather than the monotonic adjustment one might anticipate. In the analysis that follows we are concerned with the errors that are common to all cohorts in a SPA, not those that arise from the difficulties in estimating the fishing mortality in the final year.

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Analysis

Mertz and Myers (1996) showed that the population equation can be expressed as a generalized cohort analysis and from this follows a generalized catch equation. The generalized cohort analysis is given by (a related equation may be found in Hiramatsu 1995):

$$(1) \quad N_{i+1} - e^{-M} N_i = -\gamma_i(M) C_i$$

where N_i is the number of fish in the cohort in year i, C_i is the catch in year i, M is the natural mortality rate, and

$$(2) \quad \gamma_i(M) = \frac{e^{-M} \int_0^1 e^{Mt} C_i'(t)\,dt}{C_i}$$

Here, $C_i'(t)$ represents the instantaneous catch per unit time in year i, $t = 0$ represents the beginning of any year, and $t = 1$ its end. (Note that $C_i = \int_0^1 C_i'(t)\,dt$.) Mertz and Myers (1996) showed that, corresponding to eq. 1, there is a generalized catch equation given by

$$(3) \quad C_i = \gamma_i(M)^{-1} N_i (e^{-M} - e^{-(F_i+M)})$$

where F_i is the fishing mortality for year i, which is implicitly defined by $N_{i+1} = N_i \exp[-(F_i + M)]$. If fishing mortality does

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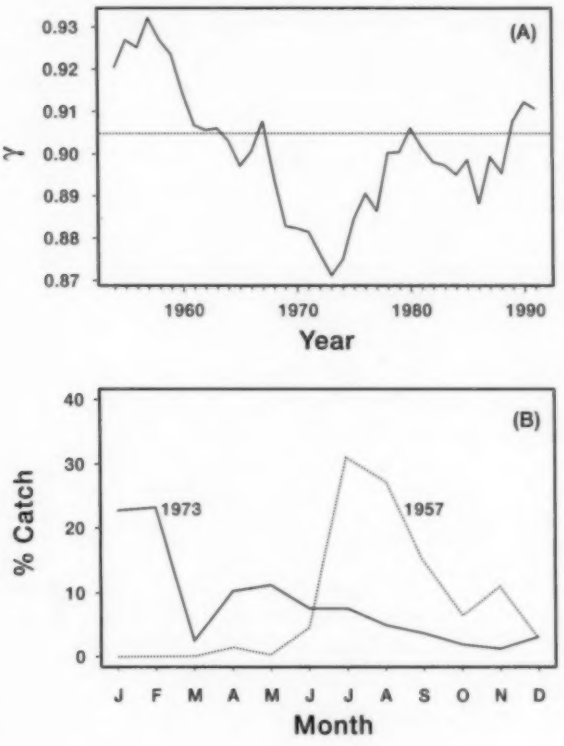
Fig. 1. (A) The factor $\gamma_i(M)$ calculated from eq. 2 using monthly catch data for northern cod, i.e., cod off Labrador and the northern Grand Banks. The dotted line is Pope's approximation. (B) Monthly catches expressed as percentages of the total for the year at the maximum and minimum $\gamma_i(M)$. Note that this figure in Mertz and Myers (1996) had the labels "1957" and "1973" reversed.



not change within a year, the standard Baranov result can be recovered from eqs. 2 and 3 (in other words, Baranov is a special case of this generalized cohort analysis; Mertz and Myers 1996).

This formulation is preferable because any pattern of seasonal fishing can be treated as a special case. For our purposes, it leads to a simple formulation in which different errors can be understood without resorting to complicated approximations, iterative solutions of the Baranov equation, or simulations.

The quantity $\gamma_i(M)$ may be analytically calculated when C_i' has a simple seasonal dependence. For example, if one has a pulse fishery at time $t = \alpha$ ($0 \le \alpha \le 1$), then $\gamma_i(M) = \gamma(M) = (e^{-M(1-\alpha)}$. For $\alpha = 0.5$, one recovers the classical cohort analysis of Pope (1972). More generally, $\gamma_i(M)$ may be computed numerically, from eq. 2, using monthly catch-at-age data. Here we are tacitly assuming that the seasonal pattern of catches for any given age is adequately represented by the seasonal pattern of the age-aggregated catch, an assumption that will generally be very accurate (Mertz and Myers 1996, p. 161). If M is not large the γ_i values show great interannual stability. This is indicated in Fig. 1, the case of northern (Northwest Atlantic Fisheries Organization (NAFO) area 2J3KL) Atlantic cod (*Gadus morhua*) for which the $\gamma_i(M = 0.2)$ series shows very little fluctuation despite the marked changes in seasonal catch pattern.

If the initial size of a cohort is N_1, it may be expressed in terms of the catches using the following relation, derived from eq. 1:

$$(4) \quad N_1 = \gamma_1(M)e^{M}C_1 + \gamma_2(M)e^{2M}C_2 + \ldots + \gamma_i(M)e^{iM}C_i + \ldots$$

Thus, N_1 is easily recovered from the catch data when M is known. In practice, the initial cohort size is computed using an estimate of M, which we designate as $\hat{M}$. Now, the estimate of N_1, designated as $\hat{N}_1$, is given by

$$(5) \quad \hat{N}_1 = \gamma_1(\hat{M})e^{\hat{M}}C_1 + \gamma_2(\hat{M})e^{2\hat{M}}C_2 + \ldots + \gamma_i(\hat{M})e^{i\hat{M}}C_i + \ldots$$

Constant fishing mortality

Before deriving a general expression for the error in N_1 associated with an incorrect M, we will the set the stage by examining the case of constant fishing mortality ($F_i = F$), which yields some helpful insights. In practical terms, a constant F is meaningful in the context of classical catch-curve analysis (fitting a line to logcatch vs. time, e.g., Hilborn and Walters 1992). In this case, we have $C_2 = C_1e^{-(F+M)}$, $C_3 = C_1e^{-2(F+M)}$, etc. Substituting these expressions into eq. 5 yields

$$(6) \quad \hat{N}_1 = \gamma(\hat{M})e^{\hat{M}}C_1(1 + e^{(\hat{M}-M)-F} + \ldots + e^{i[(\hat{M}-M)-F]} + \ldots)$$

It follows directly from eq. 3 that $C_1 = \gamma(M)^{-1}e^{-M}N_1(1 - e^{-F})$. We can use this expression to eliminate C_1 in eq. 6 and then sum the geometric series. The result is

$$(7) \quad \frac{\hat{N}_1}{N_1} = \frac{\gamma(\hat{M})e^{\hat{M}}}{\gamma(M)e^{M}} R(\Delta M, F)$$

where $\Delta M \equiv \hat{M} - M$, and

$$(8) \quad R(\Delta M, F) \equiv \frac{(1 - e^{-F})(1 - e^{n(\Delta M - F)})}{1 - e^{\Delta M - F}}$$

where n is the number of terms summed. The reasons for expressing eq. 7 in this form will become apparent.

The presence, in eq. 7, of the term $\gamma(\hat{M})e^{\hat{M}}/(\gamma(M)e^{M})$ can be understood from the following example. In the limit of infinite fishing mortality, $R(\Delta M, F) = 1$ and all of the discrepancy between $\hat{N}_1$ and N_1 can be attributed to the term $\gamma(\hat{M})e^{\hat{M}}/(\gamma(M)e^{M})$. Consider a pulse-type fishery (still with infinite fishing mortality) at time α (again $0 \le \alpha \le 1$). At the time of the catch, we know the exact cohort size is equal to C_1. At the beginning of the year, then, the cohort size was $e^{M\alpha}C_1$. For a pulse fishery (as noted earlier), $\gamma(M) = e^{-(1-\alpha)M}$, so that $e^{M}\gamma(M) = e^{M\alpha}$, and thus it is clear that the term $\gamma(M)e^{M}$ in eq. 7 arises from the projection of the cohort size at the time of catch back to the beginning of the year. Thus, the term $\gamma(\hat{M})e^{\hat{M}}/(\gamma(M)e^{M})$ represents the uncertainty arising from making this projection with a misspecified M. In fact this source of error is quite innocuous in that the maximum value of this term (attained when the entire fishery occurs as a pulse at year end) is given by $e^{\Delta M}$. Thus, the associated fractional error in N_1, $\hat{N}_1/N_1 - 1$, cannot be larger than $e^{\Delta M} - 1 \approx \Delta M$. (The corresponding expression for a pulse fishery at time α is $\hat{N}_1/N_1 - 1 = e^{\alpha\Delta M} - 1 \approx \alpha\Delta M$.)

Before discussing the effect of ΔM on $R(\Delta M, F)$, it must be noted that $\Delta M = 0$ does not guarantee $R(\Delta M, F) = 1$; in fact $R(0, F) = 1 - e^{-nF}$. The departure from unity represents a

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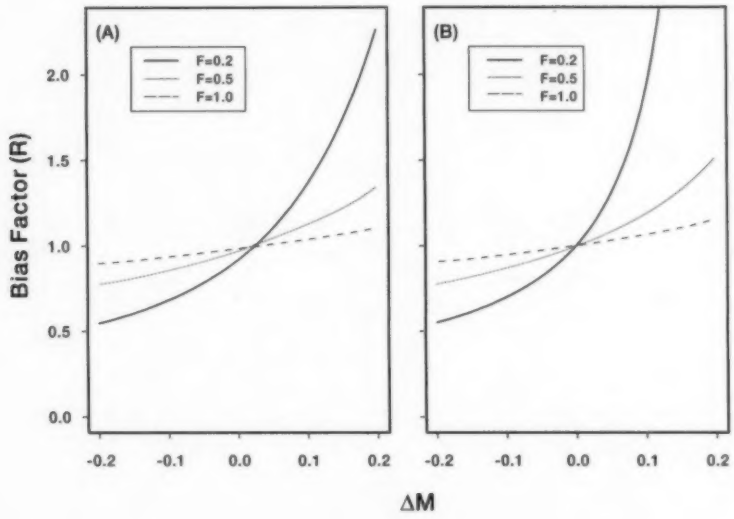
Fig. 2. The cumulative error in the ratio of the estimated to the true cohort size, $R(\Delta M, F)$, as a function of the difference in the estimated and the natural mortality, $\Delta M \equiv \hat{M} - M$, and a constant fishing mortality, F (eq. 8). The total number of catches-at-age entered to estimate the cohort size (n) is either $n = 5/(M + F)$ (A) or $n = \infty$ (B). The difference between the right and left panels is caused by different truncation effects associated with the number of ages used in the calculation.



truncation error, arising from the fact that only a finite number of annual catches are entered in the cohort analysis. This problem arises in real situations, in that reliable samples from the catches will be available only up to a given age, necessitating the assumption that there were no fish extant beyond that age (e.g., Hilborn and Walters 1992). An alternative procedure is to estimate the fishing mortality on the oldest fish and correct, using $R(0, F)$, for the truncation error (e.g., Myers and Cadigan 1995). The analysis above shows that this is only possible if ΔM is negligible; in general, one cannot separate the truncation error from the influence of ΔM (because ΔM is unknown).

The term $R(\Delta M, F)$ can inflate rapidly with increases in ΔM. Note that if $\Delta M \geq F$, then $R(\Delta M, F)$ diverges as n becomes very large (if $\Delta M < F$ then $R(\Delta M, F)$ converges to a finite value as n grows). The reason for this is clear from eq. 6: when $\Delta M \geq F$, each term in eq. 6 is greater than or equal to 1, so that the sum must diverge. A simple special case is $\Delta M = F$ for which it follows that $R(\Delta M, F) = n(1 - e^{-F}) \approx nF$; this approximation holds when F is small. Thus, if $n = 10$ and $F = 0.2$, $R(\Delta M, F) \approx 2$, which is twice the ideal. It is apparent that the most serious errors in cohort reconstruction will arise from this term.

In Fig. 2 we display $R(\Delta M, F)$ as a function of ΔM for three F values. In Fig. 2A we have specified $n = 5/(F + M)$ (so that the cohort has decayed by a factor of $e^{-5} = 0.007$) and $M = 0.2$. For comparison, Fig. 2B displays the same examples, but with $n = \infty$, for which there is no truncation effect and which is completely independent of M. It is evident that for $F = 0.5$ and 1.0 the departures of $R(\Delta M, F)$ from 1 are tolerable. For $F = 0.2$, misspecification of M can lead to severe errors in the cohort reconstruction, particularly if M is overestimated (a result foreshadowed above). Divergent behavior of $R(\Delta M, F)$ for $F = 0.2$, as ΔM approaches F, is evident in Fig. 2B. This is a manifestation of the strong dependence of $R(\Delta M, F)$ on n when $\Delta M = F$, noted above. In other words, when ΔM becomes comparable with F, the result of the cohort analysis will be very sensitive to the number of catches-at-age summed.

A general expression

The results presented above hold qualitatively when the fishing mortality is not fixed over the lifetime of a cohort. To demonstrate this we utilize the following relation, derived from eqs. 1 and 3:

$$(9) \quad C_i = \gamma_i(M)^{-1} N_1 e^{-iM} e^{-(F_1+F_2+\ldots+F_{i-1})}(1 - e^{-F_i})$$

We now enter the catches as specified by eq. 9 into eq. 5 and find

$$(10) \quad \hat{N}_1 = N_1 \sum_{i=1}^{i=n} \frac{\gamma_i(\hat{M})}{\gamma_i(M)} e^{\Delta M i} e^{-(F_1+F_2+\ldots+F_{i-1})}(1 - e^{-F_i})$$

If ΔM is comparable with the typical fishing mortality experienced by the cohort, the terms within the summation will not decrease in magnitude as i increases, leading to gross inflation of the estimate of the cohort size. This result is a close parallel to that found for the constant fishing mortality case.

Spurious time trends

The bias created by a misspecified M is dependent on the fishing mortality, and it follows that a changing F will cause a time-varying bias, which will create spurious trends in the estimates of cohort strength. Lapointe et al. (1989) have demonstrated this effect. Using the tools above we can analytically

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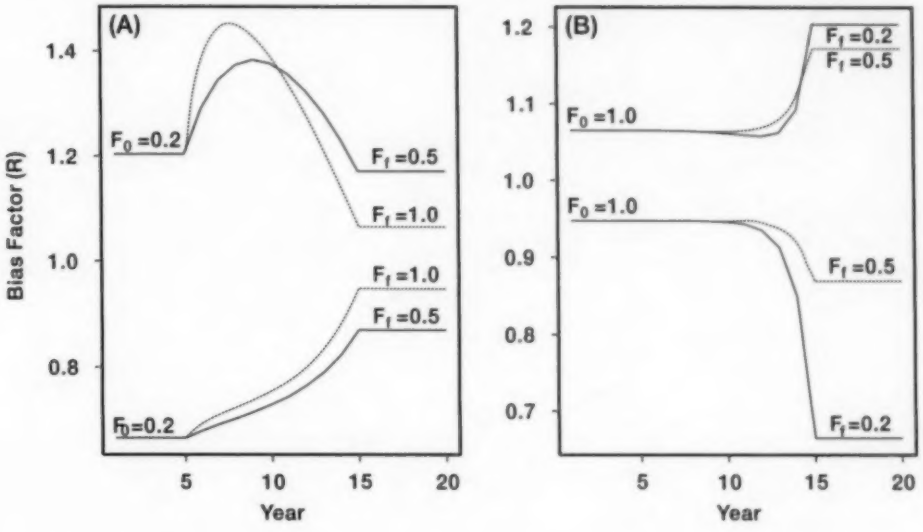
Fig. 3. (A) The effect of increasing fishing mortality ($F_0 = 0.2$) to $F_f = 0.5$ or $F_f = 1.0$ in year 5 on the ratio of the estimated to the true recruitment as manifested through $R(\Delta M, F_0, F_f)$. The top two lines correspond to $\Delta M = 0.1$, whereas the bottom two lines correspond to $\Delta M = -0.1$. Ten ages are used in the population reconstruction. (B) Same as (A) except that fishing mortality is decreased from $F_0 = 1.0$ to $F_f = 0.2$ or $F_f = 0.5$.



solve the case where a cohort is fished for j years at F_0 and for $n - j$ years at the final fishing mortality (F_f) (where n is again the range of ages entered into the cohort reconstruction). We assume that $\gamma_i(\hat{M})$ does not change substantially when F changes, an approximation that is often very accurate (Mertz and Myers 1996). Thus, the influence of the changing fishing mortality is manifested through the R term ($R(\Delta M, F_0, F_f)$, formally). Using either eq. 5 or eq. 10, we find

$$(11)\quad R(\Delta M, F_0, F_f) = \frac{(1 - e^{-F_0})(1 - e^{(\Delta M - F_0)j})}{1 - e^{(\Delta M - F_0)}} + \frac{(1 - e^{-F_f})e^{(\Delta M - F_0)j}(1 - e^{(\Delta M - F_f)(n-j)})}{1 - e^{(\Delta M - F_f)}}$$

Below, we use eq. 11 to demonstrate the effect of changing fishing mortality on cohort reconstruction.

An application of eq. 11 is shown in Fig. 3. We have considered a species for which 10 different ages are present in the fishery ($n = 10$). In each year a new cohort becomes finished, in the terminology of Evans (1996), that is, it has been fished for 10 years. The plotted R values refer to these newly finished cohorts. The fishing mortality is constant at F_0 until and including year 5, with a transition to F_f in year 6, with no further changes in F. In year 5 and all prior years, the newly finished cohorts have all been fished at F_0, i.e., $j = 10$ in eq. 11. In year 6, the newly finished cohort has been fished at F_0 for 9 years ($j = 9$), and at F_f for 1 year ($n - j = 1$). In year 15 and beyond, $j = 0$, and each newly finished cohort has been fished entirely at F_f.

In the examples plotted in Fig. 3, R exhibits a time trend as it adjusts from the first level of fishing to the second. Since R gives the bias in the cohort reconstruction, it is evident that the bias will vary temporally, which will introduce spurious trends into the estimates of cohort strength. (Strictly, R represents the most important source of bias, rather than the entire bias.) Particularly interesting is the case where ΔM is positive and fishing mortality increases; the bias actually increases before declining to the value appropriate to F_f. Thus, the trend induced by changing bias need not be a simple monotonic adjustment to the level set by the final fishing mortality. Note that an analogous result was obtained by Hilden (1988) for cases in which unfinished cohorts are reconstructed with a tuned virtual population analysis. Our analysis has shown that these effects exist even when a tuning procedure is not applied (that is, when only finished cohorts are dealt with).

Discussion

We have dealt with one aspect of the errors arising in SPA, that due to misspecification of M. The matter of estimating the size of a cohort prior to its complete removal is not dealt with here. This problem requires statistical fitting of the available catch-at-age data to a suitable index, which is itself a potential source of large errors (Hilborn and Walters 1992; Myers and Cadigan 1995). We have also neglected errors in catch (e.g., Rivard 1989) and aging errors (e.g., Bradford 1991). There are, however, clear advantages to isolating the effect of misspecification of M.

Our analysis has yielded an exact, general equation for the bias incurred by misspecification of M in cohort reconstruction (eq. 10). The case of constant fishing mortality has proved to be particularly enlightening, in that the error can be segregated into easily interpretable terms, these being the truncation error, the projection error, and the cumulative error (that resulting from the sum over ages). Finally, we showed that there are unanticipated aspects of the trends in bias arising from a changing fishing mortality in confluence with a misspecified M. We briefly discuss the impact of these error influences below.

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The truncation error will be harmless for most fish stocks. It is potentially correctable but only if one has confidence that ΔM is small so that $R(0, F)$ can be used to make the correction.

The projection error results from transposing the population estimate from the time of the catch to the beginning of the year. As noted above this error, in fractional terms, is given by $\hat{N}_1/N_1 - 1 = e^{\alpha\Delta M} - 1$ for a pulse catch at time α (which lies in the interval 0–1). Because Pope's (1972) cohort analysis is often adequate for population reconstruction and because it is equivalent to $\alpha = 0.5$, the projection error will typically be $e^{0.5\Delta M} - 1 \approx 0.5\Delta M$. Thus, it is unlikely that the projection error will exceed 10%.

The summation over ages in the cohort reconstruction can lead to a serious cumulative error when there is an overestimate in M which is comparable with the fishing mortality. In fractional terms, the severity of the error when M is underestimated is not as great, but it can nevertheless be appreciable. Based on our analysis (Fig. 2 in particular) we would suggest that $|\Delta M| \leq 0.5F$ is necessary for accurate cohort reconstruction. This implies that stocks that experience low fishing mortalities, in the range of 0.1–0.2, are particularly vulnerable to gross errors of estimation of the cohort size.

If fishing mortality is constant, the errors referred to above may not be very detrimental, in that the estimate of cohort size will simply be distorted by a constant factor. (We are assuming that natural mortality is constant.) If one is analyzing time series of log-transformed recruitment from cohort analysis, the error will simply appear as a constant offset and will not influence trends or estimates of the standard deviation of the log-transformed recruitment, a useful index of recruitment variability.

If fishing mortality does vary appreciably, then the situation is more complicated. The bias engendered by misspecification of M will vary temporally, introducing deceptive trends into the reconstructed series of recruitments. The artifactual trend may contain a hump, rather than being a monotonic adjustment from one level of bias to another, a somewhat surprising result.

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Spatial differences in the productivity of American lobster in Nova Scotia

Robert J. Miller

Abstract: Lobster production per unit area of habitat varies greatly from location to location. Several physical and biological variables have previously been offered as explanations. In this study the density of ovigerous females (a proxy for egg production), postlarvae, and recruits to the fishery were measured in seven adjacent areas spanning 190 km of Nova Scotia coast. The ranking of these densities among areas persisted from year-to-year, showing consistent spatial differences in productivity. A high spatial correlation between postlarvae and fishery recruits implicated postlarvae as the proximate regulator of lobster yields. Further correlation showed that egg to postlarval survival, and not egg abundance, was related to fishery recruits. Implications for fishery management are (*i*) enhancement of any life-history stage between postlarvae and fishery recruits should increase fishery yields and (*ii*) very different levels of egg production may be necessary to maintain an equal level of fishery recruits among areas. Larval survival was highest in areas where hatching occurred late in the season; this differs from previous suggestions that early hatching promotes survival.

Résumé : La production de homards par unité de surface d'habitat varie grandement d'un endroit à l'autre. Plusieurs variables physiques et biologiques ont été évoquées antérieurement comme explications. Dans la présente étude, la densité des femelles ovigères (un indicateur de la production d'oeufs), les poissons au stade post-larvaire et les recrues pour la pêcherie ont été mesurés dans sept endroits adjacents s'échelonnant sur 190 km de la côte de la Nouvelle-Écosse. Les densités observées dans les différentes régions ont conservé le même rang d'une année à l'autre, ce qui indique des différences spatiales cohérentes pour ce qui est de la productivité. Une forte relation spatiale entre les poissons du stade post-larvaire et les recrues à la pêcherie a fait ressortir les poissons post-larvaires comme étant le régulateur immédiat des rendements de homards. Une autre corrélation a montré que la survie du stage d'oeuf au stade post-larvaire, et non l'abondance des oeufs, était reliée au recrutement. Les conséquences pour la gestion des pêches sont (*i*) les mesures favorisant tout stade du cycle vital entre le stade post-larvaire et le stade de recrue devrait accroître les rendements de la pêcherie et (*ii*) des degrés très différents de production d'oeufs peuvent être nécessaires pour maintenir un niveau égal de recrutement dans les régions. La survie larvaire était la plus élevée dans les régions où l'éclosion survenait tard dans la saison; cette constatation diffère de conclusions tirées antérieurement selon lesquelles une éclosion précoce favoriserait la survie.
[Traduit par la Rédaction]

Introduction

Correlating abundance of prefished ages with yields to a fishery may identify where in the life history of American lobster (*Homarus americanus*) production bottlenecks occur. This, in turn, could indicate the stages where production would be profitably enhanced. For example, would increasing the minimum size taken by the fishery to increase egg production be more useful, or would adding benthic habitat to increase juvenile survival? Work to better understand limiting factors can be justified by lobster's commercial importance; the species supports the highest valued fishery in both the northeastern United States and eastern Canada (NOAA 1995; DFO 1993).

Spatial differences in lobster yields have received attention from several authors. They have associated differences with egg production, larval survival, sea surface temperature, wind and currents, and quality of bottom habitat (Table 1). However, numerical correlations with fishery yields are lacking for early life-history stages and are uncommon for physical variables, probably because data sets were not standard among areas or the areas were too few.

Most of the correlations of early life-history stages or physical factors with lobster fishery yields have used time rather than spatial series. Simple or multiple correlation coefficients were usually provided. Significant correlations have been achieved for early stage larvae, postlarvae (stage IV), 1-year prerecruits, sea surface temperature, wind, and river runoff. Studies on spatial and temporal correlations have been capably reviewed by Harding et al. (1983), Ennis (1986), Aiken and Waddy (1986), Elner and Campbell (1991), and Hudon (1994).

In this study, densities of three life-history stages were obtained from seven adjacent areas, and spatial correlations (i.e., with each area providing a data point) were calculated. From these correlations, inferences were drawn about the causes of productivity differences, where in the life history enhancement measures should be focused, and what stages might be used for temporal prediction of fishery recruits. The novel aspect of the study was the large spatial scale of sampling.

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Methods

Annual recruits to the fishery, ovigerous females, and postlarvae were measured in seven adjacent areas (Fig. 1) for 2–4 years. The 190 km of Nova Scotia coast selected for the study was known to include a wide range of lobster fishery yields (Williamson 1992).

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Table 1. A selection of studies relating spatial variation in annual landings of American lobster with physical and biological variables.

Variable	Study location	Source
Temperature	Gulf of St. Lawrence, Nova Scotia, and Bay of Fundy	Huntsman 1923 (+); Harding et al. 1983 (0)
	Gulf of St. Lawrence	Hudon et al. 1991 (+)
	Nova Scotia	Hudon 1994 (+)
Wind and currents	Nova Scotia	Dadswell 1979 (+); Robinson 1979 (+); Stasko and Gordon 1983 (+); Harding et al. 1983 (+); Watson and Miller 1991 (+/0); Hudon 1994 (+)
	Gulf of St. Lawrence	Hudon et al. 1991 (+)
Juvenile habitat	Nova Scotia	Wharton and Mann 1981 (+); Miller 1985 (0); Miller et al. 1992 (0)
	Rhode Island	Wahle 1993 (+)
Larval survival	Maritimes	Robinson 1979 (+)
Egg production	Maritimes	Wilder 1964 (0)

Note: The symbols + and 0 represent positive and no relationship, respective

Fig. 1. Location of the seven study areas.

Fishery recruits for each of the seven areas were expressed as the number taken by the fishery per square kilometre of fishing ground per year. A lobster recruit weighs 430–650 g and molts into legal size during July–September before the December–May fishing season. The commercial catch provides a good index of recruit abundance because the fishery removes about 70% of the recruits annually (Miller et al. 1987). Using the length–frequency of lobsters landed, as measured twice annually in representative ports, a length–weight regression, and the total weight of lobsters landed, the number caught per millimetre size-class could be calculated. The 81–92 mm size-classes were then summed for the number of recruits. This calculation was carried out for each of the 1988–1989 through 1991–1992 seasons, a period of stable landings.

To estimate the size of the fishing grounds, 45 fishers representing all seven areas were asked to describe where they and their neighbors fished. Fishing locations were then divided into depth zones of <36 m and >36 m, and the sizes of these zones was measured from nautical charts. Although the deep zones in some of the seven areas were large, tagging studies (R.J. Miller, unpublished) and fisher interviews indicate they were, in the main, used by a small fraction of the lobsters for overwintering. The seven areas are the smallest coastal divisions for which annual landings are reported.

The mean number of ovigerous females taken by fishers per 100 trap hauls was used as an index of egg production. During May of 1991, 1992, and 1993, 53–61 fishers, located throughout the seven study areas, kept daily records of ovigerous females caught and the number of traps hauled. This sample included about 200 000 trap hauls per year. Lobsters carry their eggs on the underside of their abdomen for nearly a year. Ovigerous females are most abundant in May catches, and the eggs do not hatch until June–September (Ennis 1975; Campbell 1986). Because ovigerous females cannot be legally retained, they are not depleted by the fishery. Fecundity per female was not compared among the seven areas, but mean size at maturity, the main factor detemining egg production in heavily fished populations (Campbell and Robinson 1983), was similar (98 and 95 mm carapace length, respectively) on fishing grounds about 60 km to the east (Watson 1988) and west (D.S. Pezzack, Halifax Fisheries Research Laboratory, personal communication) of the study area.

Postlarvae are the fourth and last stage in the water column as well as the first stage to take up a benthic habit. Thus, most of the planktonic mortality and drift occurs in earlier stages (Scarratt 1964).

Postlarvae were sampled in the top 40 cm with a plankton net. The net was 2.4 m wide, 0.5 m deep, and 4.5 m long made of 1.3 mm square mesh. It was bow mounted and pushed by a 5.5 m long outboard skiff at a speed of 1.4 m/s. Distance was measured with a flow meter mounted in the net. A typical sample filtered 640 m^3 representing 1600 m^2 of sea surface. The net tracked the sea surface well because the waves were usually longer than the boat. Large areas could be sampled because the boat moved quickly between stations and was trailered between coastal locations. The 40-cm sampling depth was thought to capture most postlarvae because they have rarely been found far from the surface (Harding et al. 1987).

Jellyfish and seaweed were removed from samples, rinsed with a pump and seawater, and discarded at sea. The remaining sample was preserved in bottles, and the larvae were counted in the laboratory. The efficiency of the rinsing procedure was tested for smaller, stage I larvae. During seven tows a known number of live larvae and a few kilograms of seaweed were added to the mouth of the net; 111 of 131 (85%) were recovered in the samples.

Trial sampling in 1989 showed larvae to be scarce near upwind shores, as reviewed by Fogarty (1983), and in small bays. Therefore, sampling was conducted in large bays and on the open coast avoiding upwind shores but otherwise at arbitrary locations within 2 km of land and a minimum of 2 km apart.

Larval sampling continued from early July through early September in 1990 and 1991. Each of the seven areas was divided into two to four subareas. Each subarea was sampled two to four (usually

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three) times per season with a median of six samples per subarea per date. Because sampling was too infrequent to calculate annual postlarval production (Scarratt 1964), the highest mean density for a subarea on any date was used as the abundance index for that subarea. Subarea means were averaged within each area. These area means were based on 8–22 samples in 1990 and 14–23 samples in 1991; a total of 351 samples were taken in 1990 and 445 in 1991.

It is assumed that postlarvae were present for long enough that major peaks in abundance were not missed. Postlarvae were present on more than one sampling date in a subarea, e.g., in the westernmost subarea of area 1 in 1991, densities were 0.3/1000 m^2 on 6 August, 3.4/1000 m^2 on 22 August, and 3.3/1000 m^2 on 10 September. Other studies have found them abundant in the plankton for 3–8 weeks (Scarratt 1964; Stasko and Gordon 1983; Hudon et al. 1986; Incze and Whale 1991). Furthermore, larval hatching within a lobster population is not synchronous (Ennis 1975; Campbell 1986) and can continue for 6–8 weeks, meaning that overlapping broods are present in the plankton.

The abundance of each life-history stage was measured as a density and was assumed to be an index of annual productivity. A mature female produces at most one clutch of eggs per year, and because each trap fishes a prescribed area (Miller 1990), the unit of 100 trap hauls provided the spatial dimension. Fishery recruits are by definition an annual crop and are expressed as number per sqaure kilometre of fishing ground. Postlarval abundance was measured as number per 1000 m^2, and the highest mean density for any sampling date in a subarea was assumed to be representative of annual production. Because densities of each of these three life-history stages varied widely among the seven areas within years (recruits by a factor of five, postlarvae 10 times, and ovigerous females by a factor of two; Fig. 2), precise measures of abundance were not necessary to detect spatial correlations.

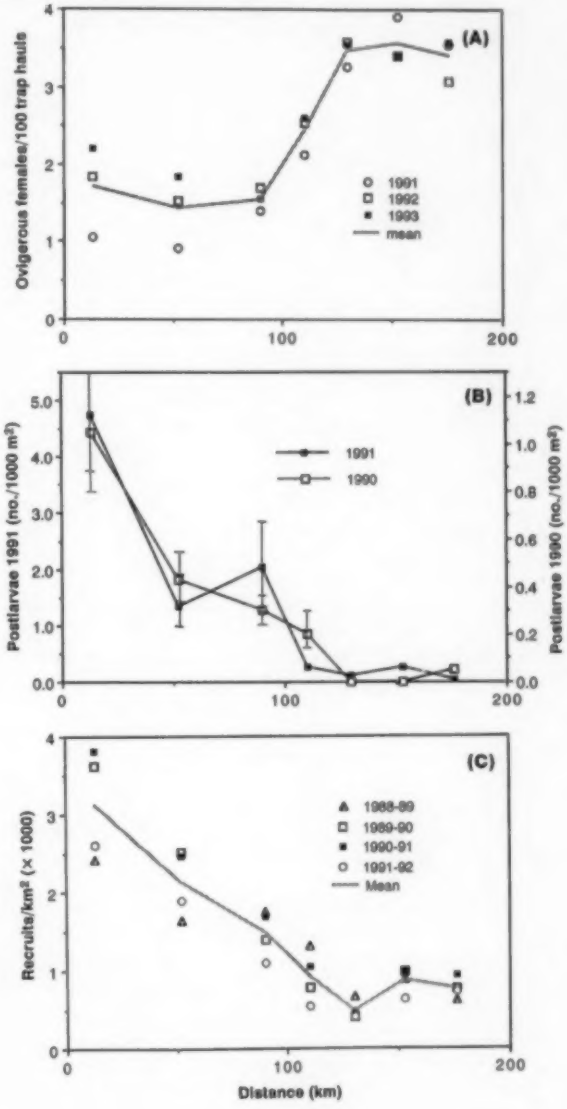
Stage I larvae (the first stage after hatching) were sampled at the same time as postlarvae, but because the stage duration is only 3–6 days (Templeman 1936), sampling was too infrequent to use density as an index of abundance. Collections were used only to determine the date of first hatching in the seven areas.

Results and discussion

For all three life-history stages, area-to-area trends in abundance were consistent among years. Ovigerous female abundance was low in areas 1, 2, and 3 relative to areas 5, 6, and 7 in all 3 years (Fig. 2A). No small locations of unusually high density were identified, as occurred in the Iles-de-la-Madeleine (Munro and Therriault 1983) and Bay of Fundy (Campbell 1990). In both 1990 and 1991, postlarval abundance decreased from areas 1 to 4 and was low in areas 5–7 (Fig. 2B), even though the annual means differed by a factor of four. Recruits to the fishery showed the same spatial trend as postlarvae (Fig. 2C) for all 4 years of observations. In fact, the ranking among areas for total landed weight has remained about the same since the 1950s (Williamson 1992). Thus, the spatial relationships have temporal stability, i.e., lobster productivity is area specific.

Postlarvae were highly correlated with fishery recruits spatially for both years of larval collection (Table 2), that is, density of both stages were high in the west and decreased markedly to the east. When recruit density was calculated using the entire fishing area rather than the prime area of <36 m, the correlation was only a little lower. We can assume negligible movement of benthic-phase lobsters among the areas. Tagging studies in area 2 (Miller et al. 1989) and area 6 (R.J. Miller, unpublished) showed longshore movement of less than 5 km for near-recruit sizes (see also review by Lawton and

Fig. 2. Abundance of three life-history stages in areas 1 through 7 proceeding from left to right: (A) ovigerous females, (B) postlarvae (error bars are +1SE calculated from values of $\ln(X + 0.01)$ then antilogged), and (C) fishery recruits.



Lavalli 1995). These correlations suggest the absence of a density-dependent bottleneck between postlarvae and recruits, which would have resulted in an asymptotic relationship at the higher densities of postlarvae.

In different analyses of the same 15-year time series of larval collections in Northumberland Strait, Scarratt (1973), Robinson (1979), and Harding et al. (1982) all found significant correlations between production of postlarvae and fishery yields. However, Fogarty (1983) and Fogarty and Idoine (1986) presented asymptotic relationships from additional treatments of the same data and suggested that habitat imposed a density-dependent limitation on fishery yields. In the late

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Table 2. Table of correlations (r^2) among postlarvae, ovigerous females, and recruits to the fishery, and the ratio of postlarvae/ovigerous females (an index of larval survival) ($n = 7$ areas).

	Postlarvae		Recruits (4-year mean)	
	1990	1991	<36 m	All depths
Postlarvae				
1990			0.94**	0.82**
1991			0.87**	0.81**
Ovigerous females				
1991		–0.51		
3-year mean	–0.53	–0.47	–0.60*	–0.41
Mean ratio of postlarvae/ovigerous females				
1990			0.97**	0.86**
1991			0.89**	0.81**

*$P < 0.05$.
**$P < 0$.

1980s, landings from western Northumberland Strait doubled from the time of Scarratt's study 20–30 years earlier (Williamson 1992). If carrying capacity had been limiting, it moved to a higher level.

Spatial correlation, as shown in this study, may be useful for identifying an early life-history stage suitable for predicting fishery recruits after an appropriate lag, i.e., a temporal prediction. In a study of spatial correlation the areas to be sampled can be chosen to include a wide range of abundance of fishery recruits, thus increasing the chance of demonstrating a correlation if one exists. On the other hand, after a long-term study of temporal correlation the investigator may find that the abundance of the stage to be predicted changed too little to establish a correlation.

Spatial is not a certain analog of temporal correlation, however. If a density bottleneck for a stage (e.g., a scarcity of shelter for juveniles) occurred in only some of the areas selected for the spatial correlation, then it could mask a correlation in the other areas. Although, if a spatial correlation can be demonstrated, I expect that a temporal correlation could also be demonstrated.

Ovigerous female abundance, a proxy for egg production, was not correlated with postlarvae in the seven areas (Table 2). This was so whether 1991 ovigerous females were compared with 1991 postlarvae, or whether the 3-year mean density of ovigerous females was compared with each year's larval collection. Reasons for this lack of correlation that could have operated during the three intervening larval stages are density-dependent mortality, larval drift among the seven areas, and density-independent mortality.

I dismiss density-dependent mortality because larvae are too dilute to compete for resources or attract predators. The maximum mean density of stage I plus postlarvae collected in any subarea was 2.7 individuals/100 m^3 or 40 mg dry weight/100 m^3 (based on sizes of wild postlarvae; Juinio and Cobb 1994). Stages II and III were not collected because they remained too deep for my sampling gear (Harding et al. 1987). However, even increasing the observed larval densities a few-fold would not likely have an impact on their zooplankton prey or alter the behaviour of a predator.

Drift of larvae from the area where they hatched into another area before they were sampled as postlarvae cannot be ruled out as contributing to the lack of correlation between eggs and postlarvae. Some of the postlarvae collected in western areas might have drifted in wind-induced surface currents from Browns Bank, centered 160 km southwest of area 1. However, most probable directions of drift were north, towards the Bay of Fundy, and east, offshore on the Scotian Shelf (Harding and Trites 1988; Watson and Miller 1991). Longshore drift between the seven sampled areas is also possible, but these areas were not small (20–45 km wide) and included numerous bays. Also, the dominant westward-flowing Nova Scotia current is weakest during the July–August larval period and has a mean transport near zero near shore (Drinkwater 1979).

Different density-independent larval mortality among areas is the explanation favored here for the lack of egg–postlarval correlation. The mortality differences could be caused by any of a variety of environmental influences, such as advection offshore away from lobster habitat, starvation, or predation. Food or predators can limit survival of larvae and survival can still be density independent if the population of lobster larvae does not alone deplete the food supply or attract predators. Even though larval survival would vary from place to place and year to year, on average, more eggs would result in more postlarvae.

Because of the large effect of environmental "noise" on larval survival, temporal correlations of eggs or early larvae with subsequent life-history stages have been difficult to demonstrate for deacapods (Fogarty and Idoine 1986; Phillips 1986; Botsford 1992; Pollock 1993; Lipcius and Cobb 1994) and fish (Gulland 1977; Leggett and DeBlois 1994). Furthermore, Wilder (1964) showed no relationship between egg production and the size of the commercial lobster stock among three fishing ports in eastern Canada, and Ennis (1991) showed a weak negative correlation between egg production and fishery recruits (lagged 9 years) in a 15-year time series for a population in southern Newfoundland.

Time of hatching may affect the density-independent mortality hypothesized above. The density of both postlarvae and fishery recruits were lowest in the three eastern areas (Fig. 2). These areas warm faster (Drinkwater and Trites 1987; Hudon 1994), and larvae were collected 2–5 weeks earlier than in the four western areas (Fig. 3). Had saampling begun earlier, the time difference may have been even greater because larvae were usually present in the east on the first sampling dates. Thus, the apparent east–west difference in larval survival may be associated with a different match with environmental controls (e.g., food, predators, advection, depth of seasonal thermocline). Based on calculations of degree-days needed for larval development, other authors have postulated that late hatching leads to lower larval survival (Scarratt 1964; Caddy 1979; Harding et al. 1983).

The area of transition from high to low lobster production noted here is near but not the same as that noted by others. Areas 5, 6, and 7 are similar to one another in all parameters (Figs. 2 and 3) and different from the other areas. By analyzing long histories of lobster landings over larger areas than included in this study, Harding et al. (1983) and Campbell and Mohn (1983) designated area 2 as a transition zone between the Gulf of Maine and eastern Nova Scotia. Hudon (1994) suggested the break in similar landings trends occurred at the border of areas 2 and 3.

Recruit density, that is, productivity of a lobster fishing ground, may be influenced by the survival rate of larvae. We

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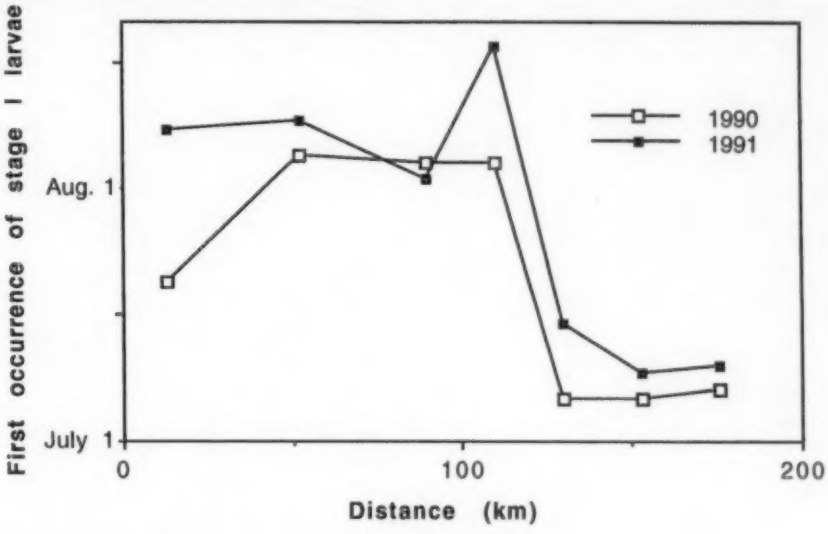
Fig. 3. Date of first occurrence of stage I larvae in the seven areas.



saw above that postlarvae were highly correlated with recruits. The ratio of postlarvae/ovigerous females, which is an index of larval survival, has a marginally higher correlation with recruit density (Table 2). If larval survival varied independently of recruits, then we would expect a lower correlation. From inspection of the time series of larvae and commercial landings in Northumberland Strait (Scarratt 1964, 1973), Robinson (1979) also concluded that fishery recruits may be correlated with larval survival.

The principal conclusions of this study, that lobster productivity is area specific, that postlarvae were highly correlated with fishery recruits, and that survival through the larval stages may cause differences in productivity among areas, have implications for managing the lobster fishery: (*i*) stocking postlarvae or early benthic stages, or enhancing their survival on the fishing grounds, should increase the number of fishery recruits up to the point of exceeding habitat carrying capacity; (*ii*) if mortality from eggs to postlarvae and from postlarvae to fishery recruits is density independent as suggested here, then increasing egg production (with a larger minimum legal size, a legal maximum size, closing areas to fishing, or reducing exploitation rate; Campbell 1986) will, on average, increase the number of recruits. The link may be difficult to demonstrate, however, because of the effects of environmental noise on larval survival; (*iii*) if survival through the larval stages is a major cause of spatial differences in recruitment to the fishery, then different areas will require different levels of egg production to yield the same level of fishery recruits. Thus, recently recommended conservation targets of a uniform 10% of virgin egg production for the United States (NOAA 1994) and 5% for the Canadian lobster fisheries (Fisheries Resource Conservation Council 1995) may be solutions that are too simple.

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Detection of migrating salmon in the Fraser River using 100-kHz sidescan sonars

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Abstract: During a 10-day period in September 1995, field trials were conducted in the Fraser River near Mission, B.C., to evaluate 100-kHz side-looking sonars for salmon detection. A two-element sidescan installation looking transverse to the river flow detected echo traces due to salmon at ranges up to 250 m in water 6–13 m deep. The salmon target trajectories were identified and counted against a background of acoustic reverberation from the river surface and bottom sediments. Occasional, strong interference from boat wakes and wind-induced surface waves and bubbles was observed. Fish echo trajectory analyses allowed extraction of upstream and cross-stream swimming speeds. For some targets acoustic multipaths from surface and bottom reflections were distinguishable from direct echoes at ranges up to 150 m. Acoustic models of surface and bottom boundary backscattering, combined with bubble layer scattering and attenuation under breaking wave conditions, were able to match the observed background echo levels. Comparisons of model and data predict that under ideal conditions fish detection should be possible at ranges up to 250 m, but under windy conditions detection was limited to less than 70 m.

Résumé : Au cours d'une période de 10 jours en septembre 1995, des essais sur le terrain ont été réalisés dans le fleuve Fraser près de Mission, C.-B., pour évaluer l'efficacité de sonars à balayage latéral de 100 kHz pour la détection du saumon. Une installation à balayage latéral à deux éléments sondant selon un axe transversal par rapport à l'écoulement du fleuve a permis de détecter des échos de saumons à des distances allant jusqu'à 250 m dans des eaux variant de 6 à 13 m de profondeur. Des trajectoires des cibles (saumon) ont été déterminées et dénombrées par rapport à un fond de réverbération acoustique sur la surface de l'eau et les sédiments de fond. On a observé une forte interférence occasionnelle causée par les sillages des bateaux ainsi que par les vagues et les bulles produites à la surface par le vent. L'analyse des trajectoires des échos de poissons a permis de déterminer les vitesses de nage vers l'amont et transversalement par rapport à l'écoulement. Pour certaines cibles, les trajets acoustiques multiples provenant de la réflexion depuis la surface et le fond pouvaient se distinguer des échos directs à des distances allant jusqu'à 150 m. Les modèles acoustiques de rétrodiffusion depuis la limite de la surface et du fond, combinés à la diffusion par la couche de bulles et à l'atténuation dans des conditions de vagues déferlantes, pouvaient correspondre aux niveaux d'écho observés comme bruit de fond. Les comparaisons avec le modèle et les données permettent de dire que dans des conditions idéales, la détection des poissons devrait être possible à des distances allant jusqu'à 250 m, mais lorsque le temps est venteux, la détection est limitée à moins de 70 m.
[Traduit par la Rédaction]

Introduction

Horizontally oriented, narrow-beam sonars have recently been developed for detection of fish in shallow rivers and lakes (e.g., Gaudet 1990; Kubecka et al. 1994; Duncan and Kubecka 1996). In such environments, the use of conventional vessel-mounted echo sounders is generally limited owing to the small sonar sampling volume beneath the boat and boat-avoidance behaviour by the fish. In a side-looking geometry, both simple and compound-beam sonar systems have the advantages of rapid scanning of the river cross section to much greater range without affecting fish behaviour. At the relatively low fish densities typical of this river, methods have been devised to estimate fish escapement based on echo counting and target duration in the beam (e.g., Kieser and Mulligan 1984; Thorne 1988; Gaudet 1990; Banneheka et al. 1995). High-resolution split-beam systems have the additional advantage of tracking fish trajectories in three dimensions (Steig and Johnston 1996; Mulligan and Kieser 1996). As a general rule in these applications, however, surface and bottom boundary reverberation has been specifically avoided by careful sonar beam steering and restriction of the operating range (typically <40 m). A limitation with this approach is that the fish targets can often be found at greater range and very close to the boundaries.

A potential solution is the use of side-looking, fan-beam sonars with beams that actually graze the surface and bottom boundaries. Sidescan sonars are commonly used for bathymetric mapping and underwater surveys, and several studies have investigated their use for fisheries surveys in the open ocean (Hewitt et al. 1976; Rusby 1977). An advantage of sidescan systems is that large proportions of the river cross-section can be insonified in a single transmission. In rivers, acoustic scanning at greater range and close to the boundaries by necessity invites backscattered interference from bottom sediments, surface roughness, breaking waves, and boat wakes. Whether using simple or compound-beam sonars, the fundamental detection efficiency at any given range will depend on the target (fish) backscatter cross section relative to the combined contributions from other volume scatterers and boundary reverberation. Acoustically, the task herein is to quantify the level of this interference and to devise methods for recognizing fish echoes against this background. One confounding issue is

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that natural fish targets exhibit a wide range of target strengths depending on size, orientation relative to the sonar beam, and swimming motions.

The site selected for this study was the Fraser River near Mission, B.C., at the same location as stock assessment operations run by the Pacific Salmon Commission (PSC). This location was selected because there was known to be an abundance of salmon during August and September, with identification of the salmon species based on fishery landings and run timing provided by the PSC. Two sidescan transducers were installed near the south river bank, looking transverse to the river flow. Utilizing the two 3° by 60° sidescan beams in both vertical and horizontal orientations, a variety of beam geometries could be evaluated.

This work will first describe pertinent acoustic scattering models for the reverberation from river bottom and surface roughness, backscatter, attenuation due to breaking wave induced air bubbles, and volume scattering. Range versus time echograms will then show examples of fish target trajectories and other features observed with the horizontally wide and narrow beams. Finally, quantitative acoustic comparisons of the fish echo strength relative to the background reverberation will demonstrate the fish detection ability and limitations of this approach.

Boundary reverberation mechanisms

To gain an understanding of limitations to fish detection at greater range it is necessary to construct an acoustic model that includes both fish targets and boundary reverberation. For a general backscatter sonar the variation of received echo (intensity) level, RL, versus range, r, can be expressed in decibels (following Clay and Medwin 1977) as

$$(1)\quad \mathrm{RL} = K + 20\log(A) + \mathrm{TVG} + \mathrm{TS} - 40\log(r) - 2\alpha r + \mathrm{BDL} + \mathrm{TL}_{\mathrm{bub}}$$

where K is the acoustic calibration constant (includes transmit source level, transducer sensitivities, receive gains, and analog to digital conversion), A is the digital echo amplitude (integrated over a pulse length),TVG is the receiver time-variable gain, TS is the effective target strength of the scattering mechanism under consideration, α is the acoustic absorption in fresh water at the transmit frequency (at 100 kHz it is 0.0026 db/m), BDL is the beam deviation loss calculated from the transducer transmit and receive beam patterns, and $\mathrm{TL}_{\mathrm{bub}}$ is the transmission loss due to air bubbles (if present). The calibration coefficient can be calculated using the backscatter from standard target spheres (following e.g., Foote et al. 1987 or Vagle et al. 1996).

Various acoustic targets and background scattering mechanisms will contribute to the total echo. The adult salmon targets that are the focus of this study have typical length near 50 cm, and their mean target strength at 100 kHz reaches a maximum at lateral incidence in the range –25 to –32 db (Thorne 1979; Dahl and Mathisen 1983). There are, however, significant variations due to fish size, direction of insonification, curvature, and swimming activity levels. For comparison purposes herein, a uniform fish TS of –30 db will be used.

In this riverine situation, the sidescan beams intersect the surface and bottom boundaries at small grazing angles, typically less than 10°. For acoustic backscatter from both air–water and water–sediment interfaces, the effective target strength (db) is strongly dependent on grazing angle and interfacial roughness and increases proportional to the insonified surface area, i.e., as (for low grazing angles):

$$(2)\quad \mathrm{TS}_{\mathrm{bndry}} = 10\log[S_a(\phi)] + 10\log(\tfrac{1}{2}c\tau r\theta)$$

where $S_a(\phi)$ is the backscatter cross section per unit area at grazing angle ϕ, c is the sound speed, τ is the pulse length, and θ is the equivalent transducer beam width parallel to the boundary. Extensive theoretical investigations of the boundary scattering cross section have been conducted since the 1960s, for both ocean surface and sediment reverberation (see Fortuin 1970 and McDaniel 1993 for literature reviews). The general approach involves modifications to the fundamental Bragg backscattering equations to encompass various models of surface roughness and to include effects of tilting of the surface at larger spatial scales and shadowing at low grazing angles. Because of the common occurrence of near-surface air bubbles in marine experiments, there are only a few uncontaminated measurements of surface backscatter at high acoustic frequencies and low grazing angles (Garrison et al. 1960; Reeves et al. 1969; Gardner 1973). These measurements yielded (for winds <10 knots (1 knot = 1.852 km/h)) $10\log[S_a(\phi)]$ near –35 db at grazing angle of 10° but diminishing roughly as at lower grazing angles. Similarly for natural silty–sand sediments, high-frequency measurements at low grazing angles by McKinney and Anderson (1964) and Wong and Chesterman (1968) yielded $10\log[S_a(\phi)]$ near –36 to –40 db, roughly independent of grazing angle up to 10°.

For frequencies greater than approximately 20 kHz, backscatter from microscopic air bubbles created by breaking surface waves and (or) boat wakes becomes dominant (McDaniel 1993; Trevorrow et al. 1994). These bubbles have a broad size distribution, with radius, a, in the range 10–200 μm and with the size spectrum decreasing approximately as $a^{-4.5}$ from a peak near 20 μm (Hall 1989). With sufficient breaking wave activity these bubbles will form a roughly uniform near-surface layer that can be modeled crudely using a vertical exponential decay with depth scale in the range 0.2 to 0.5 m (Crawford and Farmer 1987). This simplified bubble size and depth variation is thus

$$(3)\quad n(a, z) = n_0 \cdot (a/a_{\mathrm{peak}})^{-4.5} \exp(-z/L), \qquad \text{for } a > a_{\mathrm{peak}}$$
$$n(a, z) = n_0 \exp(-z/L), \qquad \text{for } a < a_{\mathrm{peak}}$$

where n_0 is the surface bubble spectral density, a_{peak} is the peak radius, z is depth (positive down), and L is bubble depth scale. Using this size distribution, the bubble extinction and total scattering cross sections per unit volume, $S_e(f,z)$ and $M_v(f,z)$ (units of m^{-1}), can be calculated using formulations contained in Clay and Medwin (1977). The volumetric extinction cross section, $S_e(f,z)$, can be used to calculate the excess acoustic attenuation due to bubbles (db), given by the depth-integrated path through the bubble layer, i.e., as

$$(4)\quad \mathrm{TL}_{\mathrm{bub}}(f, \phi) = \left(\frac{8.68}{\sin\phi}\right)\int_0^\infty S_e(f, z)\mathrm{d}z$$

This bubble loss applies equally to backscatter from the bubble layer, surface roughness scattering and reflections, and any fish targets in the surface bubble layer. Similarly, the low-grazing angle backscatter target strength (db) of a bubble layer will be

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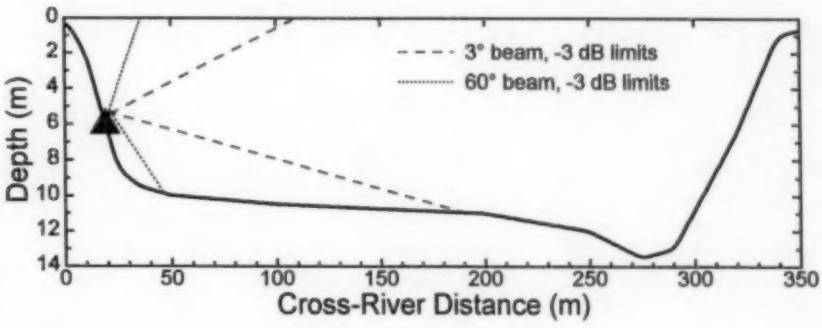
Fig. 1. Cross section of the Fraser River at the Mission, B.C., site (downstream view) along heading 323° true. The sonar tripod was deployed at 6.1 m (nominal) water depth, with the upper transducer at 4.5 m depth. Vertically wide and narrow beam limits (to –3 db) approximately as shown.



given by the depth integral of $M_v(f,z)$ multiplied by the insonified surface area of the pulse, i.e.,

$$(5)\quad TS_{bub} = 10\log\left((4\pi)^{-1}\int_0^{\infty} M_v(f,z)dz\right) + 10\log\left(\frac{1}{2}c\tau r\theta\right)$$

An important characteristic of bubble layer scatter is that for typical bubble densities and small grazing angles the attenuation generally exceeds the volume scattering terms, resulting in a decreasing echo scattering strength ($TS_{bub} - TL_{bub}$) with range even though the insonified volume increases proportional to range.

For the case of low grazing angle backscatter from a bubble layer, the vertical variations of M_v, S_e, and BDL must be included together in the calculation of the total scattering strength from the layer at any given range. This implies substituting vertical integrals from eqs. 4 and 5 into the basic model (eq. 1), which after some manipulation becomes

$$(6)\quad RL = TVG - 40\log(r) - 2\alpha r + 10\log(\sigma)$$

where

$$\sigma = \frac{1}{2}\frac{c\tau r\theta}{4\pi}\int_0^{\infty} M_v(z)\mathrm{BDL}(\phi) \times \exp\left(\frac{-2S_e(z)L}{\sin(\phi)}\right)dz$$

where the grazing angle, ϕ, inside the integral is evaluated as a function of depth. In this integral, bubble backscatter will be greater near the surface, but this is balanced by both stronger attenuation as the surface is approached and higher beam deviation loss at larger deviation angles. The beam deviation loss term is calculated from the measured transmit and receive beam patterns of the sidescan transducers. Specular surface reflection combined with backscattering from the bubble layer (focusing effects) are neglected in this formulation.

In the river there will be an additional volume scattering contribution due to suspended sediments, given by

$$(7)\quad TS_{vol} = 10\log(S_v) + 10\log\left[\frac{1}{2}c\tau r^2\psi\right]$$

where S_v is the volumetric scattering cross section (m^{-1}) and ψ is the equivalent solid angle of the transducer including both transmit and receive beam properties. For small particles, the volume scattering strength increases strongly with frequency and particle size (typically as f^4 and a^6 for wavelength >> target dimension). For example, using a sediment diameter of 0.2 mm and appropriate elasticity and density constants, the backscatter cross section at 100 kHz lies near 3×10^{-14} m^2. Assuming roughly 10^5 to 10^6 grains per cubic meter (1–10 $g \cdot m^{-3}$), this yields a volume scattering strength of –85 to –75 db re 1 m^{-1}, which is clearly not important to this study. However, at higher frequencies this might become important (e.g., at 300 kHz the scattering strength would rise to –65 to –55 db re 1 m^{-1}). In a lake or marine environment, zooplankton could replace suspended sediments as the dominant source of volume scattering, with scattering strength in the range –80 to –60 db re 1 m^{-1}, depending on frequency, species, and population density.

Instrumentation and experiments

The sidescan experiments were conducted from September 18–29, 1995. The location was roughly 25 m from the south bank of the Fraser River, approximately 2 km upstream from the bridge at Mission, B.C. (at 49°08.18′N, 122°16.50′W). Figure 1 shows the approximate river cross section as determined from echo-sounding transects of the river by the PSC boat. The underwater system consisted of two 100-kHz sidescan transducers cabled to an attending boat. A large concrete block supported an adjustable mounting tripod, which was leveled manually by divers during deployment. The depth of the top of the tripod was nominally 4.4 m, with a depth variation (mostly due to tidal effects) of roughly ±0.5 m. The two sidescans were oriented transverse to the river flow along a heading 323° true. The support boat housed the sidescan transmitter–receiver electronics (a BioSonics model 101 echo sounder), a 486PC data acquisition computer, and an electric generator.

The sidescan transducers had fan-shaped beams 3° by 60° (to –3 db), with maximum sidelobes at –24 db. One transducer was mounted with its long axis vertical (denoted V), providing a 60° wide horizontal beam only 3° in vertical extent. The other transducer was mounted with its long axis horizontal (denoted H), enabling insonification of almost the entire river cross section in a horizontally narrow beam. Each transducer could be used as either transmitter and (or) receiver, allowing four beam–geometry combinations (denoted VV, VH, HV, and HH where the first letter is the transmitter and the second

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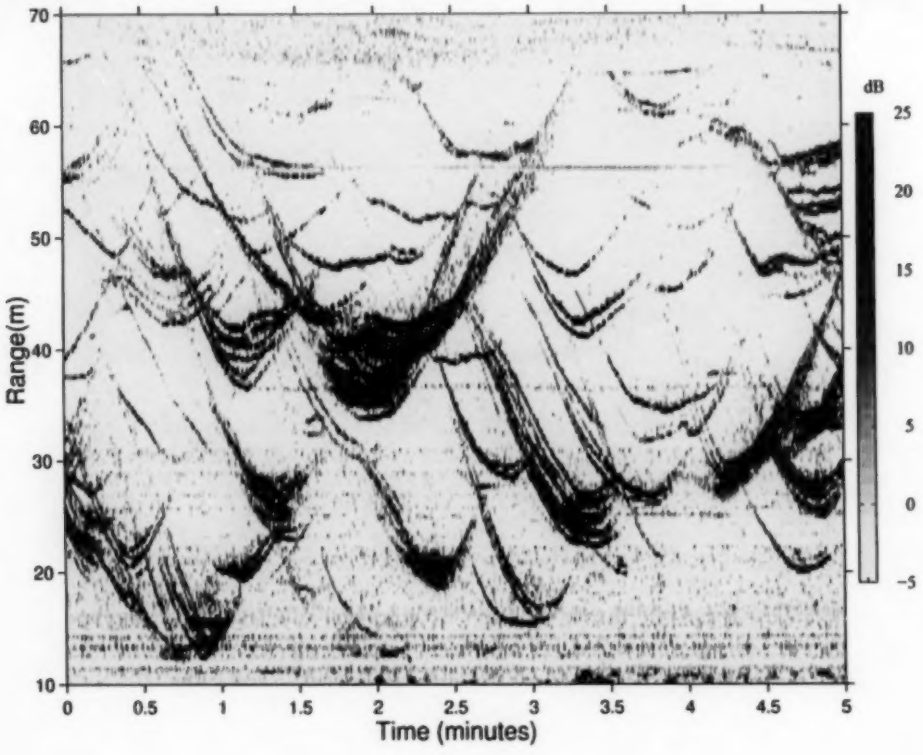
Fig. 2. Backscatter intensity (db re time-averaged intensity) echogram using the VV sidescan geometry starting 18:41:30 Sept. 24, 1995.



letter is the receiver). The intersection of the two crossed sidescan beams yielded a 3° × 3° square beam. In later analysis it became evident that the transducers were mounted with a 1.4° upward tilt, causing the vertically narrow beam to intersect the surface beyond 90 m range. The far-field distance for these transducers was approximately 5 m. The water temperature was nominally 15°C, yielding a freshwater sound speed of 1465 m/s (this was used in all calculations). Information from the PSC indicated that the salmon targets at this time were a mixture of pink (*Oncorhynchus gorbuscha*) and sockeye (*Oncorhynchus nerka*) salmon.

The transmitter provided approximately 500 W (electrical) to the transducer, corresponding to an on-axis source level of roughly 215 db re 1 μPa. A pulse length of 0.5 ms was used, yielding range resolution of 37 cm. The sidescan transmissions were triggered at 0.6 s intervals. The signal detection electronics used a 6-db pre-amplification and a 20log(r) time-variable gain (TVG) to partially compensate for geometric spreading across the river. Note from eq. 1 that a 40log(r) TVG is appropriate for individual fish echoes. However, in this application the use of a TVG greater than 20log(r) caused a blowup of the boundary reverberation terms, which have increasing insonified area with range. The received echoes were mixed down to an 8-kHz carrier, amplitude detected, then digitized at a base sample rate of 5000 samples/s with 12-bit resolution. To reduce the overall data storage requirements, successive echo amplitude pairs were averaged to yield an effective sampling rate of 2500 samples/s (29 cm sampling resolution). The backscattered amplitude versus range data was stored in 30- or 60-min (nominal) data files, collected typically two to six times daily. A total of 38 h of data under a variety of weather and flow conditions were collected. Typically the winds were light and the river surface flat calm. However, one aspect of this study was to compare performance under calm and windy conditions, and there were several periods with winds up to 10 m/s (estimated visually by the author).

After the field trials this two-element sidescan system was calibrated utilizing the backscatter from tungsten-carbide target spheres (following Vagle et al. 1996). Separate calibration runs with three different sphere radii (19.05, 20.00, 21.50 mm) were performed. The target spheres were suspended using monofilament fishing line at several ranges from 5 to 15 m along the main axis of the transducer beam. Echo intensities were averaged over 200 transmissions. By comparing the calibration results from the three spheres at multiple ranges, the accuracy in RL measurements was estimated ±0.5 db. The transducer beam patterns were provided by the manufacturer (EDO/Western Corp.).

Acoustic results

Echogram analysis

Before quantitative analysis of the sidescan echoes, it is useful to examine some of the general characteristics of the range versus time echograms collected during this study. Figure 2 shows a typical VV (monostatic wide horizontal beam) sidescan echogram taken on the evening of September 20. In this

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Fig. 3. Probability distribution of upstream swim speed from analysis of hyperbolic trajectories from 17 one-hour VV sidescan data sets, Sept. 20–29, 1995. Distribution normalized such that $\int_0^{200}\phi(u)du = 1$.

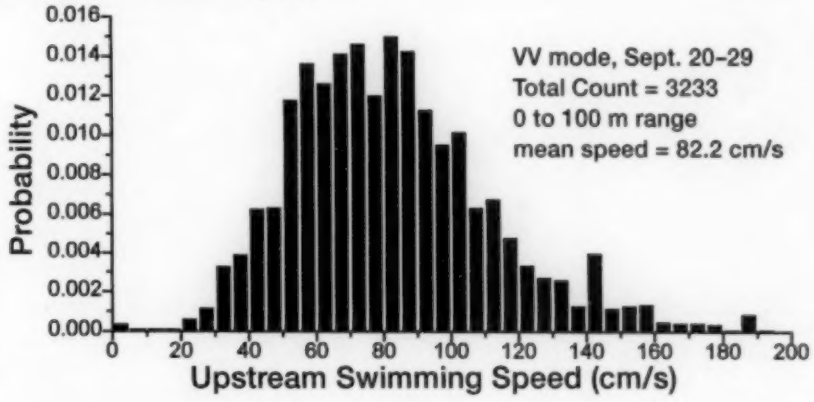

image the transient fish trajectories have been enhanced by referencing the decibel intensity scale to the time-invariant background reverberation. This processing specifically suppresses reflections from fixed targets (such as submerged logs), the river surface and bottom, and back reflections from the near river bank. This referencing also creates the weak constant range lines found in the figure. The fish target trajectories are clearly seen to ranges of at least 60 m, with duration in the beam ranging from 20 to 80 s and target counts of order 500 per h. The range limit in this example is due to reverberative interference from boat wakes and bubble scattering beyond 70 m (discussed below), created by a roughly 8 m/s wind. Many of the target trajectories show one or two ghost images, presumably owing to surface and bottom reflected multipaths. This echogram also shows overlapping trajectories and several examples of fish swarming close together such that individuals are not acoustically separable (this behaviour is common with pink salmon).

Using manual, user-interactive counting techniques, echograms such as Fig. 2 were analyzed for parameters such as fish numbers, cross-channel distributions, and swimming speeds. In this preliminary analysis, each fish target was visually recognized, then outlined by the operator (the author) using a computer mouse. This manual recognition technique allowed rejection of spurious traces due to acoustic multipaths and avoided interference from boat wakes and wind-induced surface backscatter. The analysis software then scanned each manually selected target for such variables as duration, width, maximum intensity, slope, and curvature. Finally, summary statistics over a large number of trajectories were extracted.

The wide horizontal beam (VV) geometry was useful for estimating the upstream swimming speed. Under the assumption that the fish were swimming straight upstream, each trajectory should approximate a hyperbola on a range versus time plot, i.e., as $r^2 = r_0 2 + u^2\ (t - t_0)^2$, where r is range from transducer, u is the swimming speed, and t is time, with r_0 and t_0 the point of closest approach. Approximating this VV-mode sidescan beam as an elliptical cone with horizontal extent ±30°, it can be shown that the duration of the echogram traces should limited to $<2r_0/(3^{1/2}u)$. For example, at 50 m range and swimming speed of 0.8 m/s, the trace duration will be <72 s. Examples of hyperbolic trajectories with such time scales can be found in Fig. 2. If the fish trajectory is angled partially across the stream, then the echogram trace will not be symmetric about (r_0, t_0), which routinely occurs. To extract swimming speed from this type of trace, the vertex and six other points along the trace were identified using the mouse. Then a least-squares fit of $(r - r_0)^2$ versus $(t - t_0)^2$ yielded the mean swimming speed. Note that this analysis cannot distinguish between targets traveling up- or down-stream, but information from the PSC (specifically split-beam sonar trajectories) supports the assumption that the salmon were almost entirely swimming upstream in this region of the river.

Through curve fitting to more than 3200 separate fish trajectories over the 10 days of observations, a summary histogram of swim speeds can be generated, as shown in Fig. 3. To avoid contamination from boat wakes and surface reverberation, only traces from the first 100 m range were included in this analysis. The mean upstream swimming speed was 82 cm/s (relative to the fixed sonar tripod), with approximately 73% of the fish targets swimming at speeds in the range 50–105 cm/s. With a typical river flow speed of 60 cm/s during this period, the mean fish swimming speed relative to the water was 1.4 m/s.

The narrow horizontal beam geometries (HH, HV, and VH modes) were useful for quantifying the cross-river fish distributions and transverse swimming speeds. In addition, because of the wide vertical acceptance angle of these beam geometries, acoustic reflection multipath characteristics could be examined. With a narrow horizontal beam width the fish targets appear as short linear traces, as shown with a typical VH mode sidescan echogram in Fig. 4. Similar to Fig. 2, this image has been processed to remove the time-invariant background reverberation. Similar to the wide horizontal beam geometry, these linear traces were frequently accompanied by acoustic multipaths and exhibited swarming behaviour. The duration of each trace is again proportional to the fish swimming speed and horizontal beam extent at the fish range. For example, at 100 m range the horizontal beam extent is roughly 5.2 m, so a fish swimming at 0.8 m/s would be observed for only 6.5 s (<11 pings). In further analysis of such traces, a fish trajectory was only considered valid if it was continuous for at least 4 pings (2.4 s).

The slope of the trace is a direct measure of the cross-river

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Fig. 4. Backscatter intensity (db re time-averaged intensity) echogram using the VH sidescan geometry starting 18:47:32 Sept. 21, 1995.

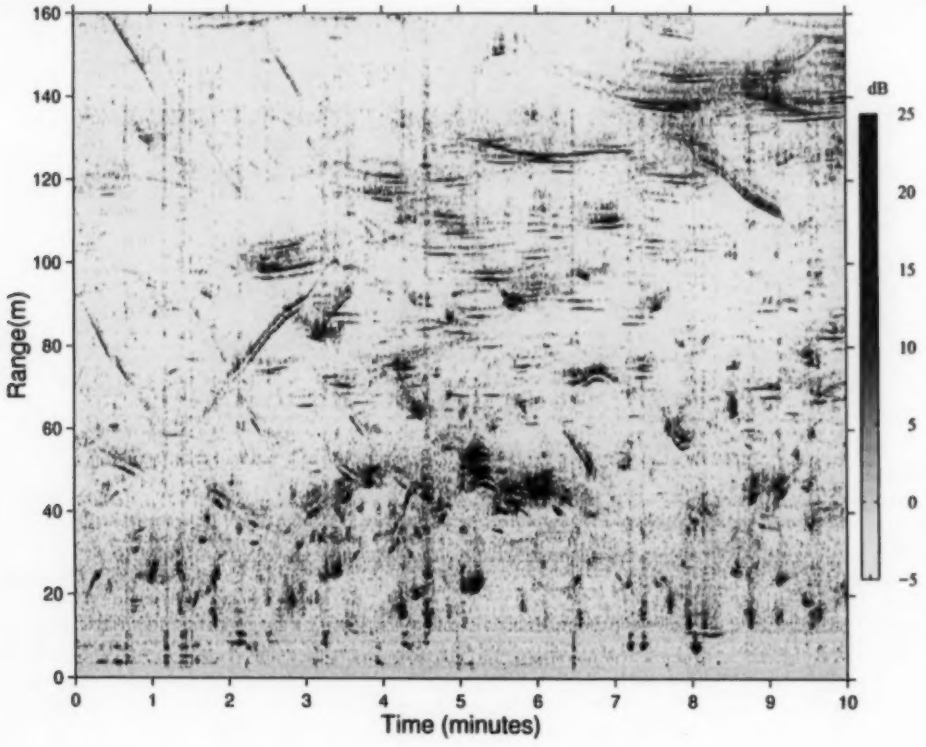
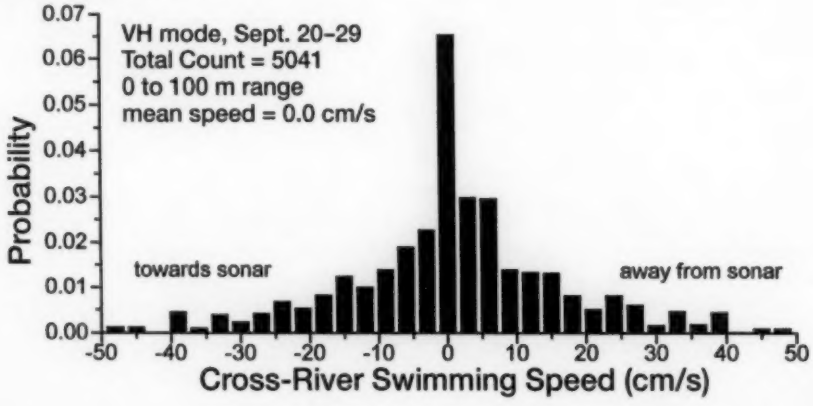


Fig. 5. Probability distribution of cross-river speed from trajectory slopes for 21 one-hour VH sidescan data sets, Sept. 20–29th, 1995. Distribution normalized such that $\int_{-50}^{50}\phi(v)dv = 1$.



velocity component ($v = \Delta r/\Delta t$). Figure 4 shows examples of fish targets swimming both towards and away from the sonar. Figure 5 shows the summary distribution of cross-river speeds taken up to 100 m range for all the VH mode data sets. From over 5000 separate targets, the mean cross-river speed was zero, with approximately 78% of fish showing transverse speeds in the range ±20 cm/s. With a mean upstream speed of 82 cm/s and a typical cross-stream speed of <20 cm/s, this implies the fish to be swimming (assumed upstream) within ±14° of the river flow direction.

A confounding feature of the river environment were surface- and bottom-reflected acoustic multipaths. Figures 2 and 4 contain several examples of target trajectories with multipath ghost images. In the VH sidescan mode, the narrow vertical beam insonified fish in the middle to upper portions of the water column only, while the wide vertical beam receiver allowed acceptance of nearly all surface- and bottom-reflected multipaths. The time delay to these multipaths is a sensitive function of fish depth, range, and (for bottom reflected arrivals) water depth. The typically observed time delay was

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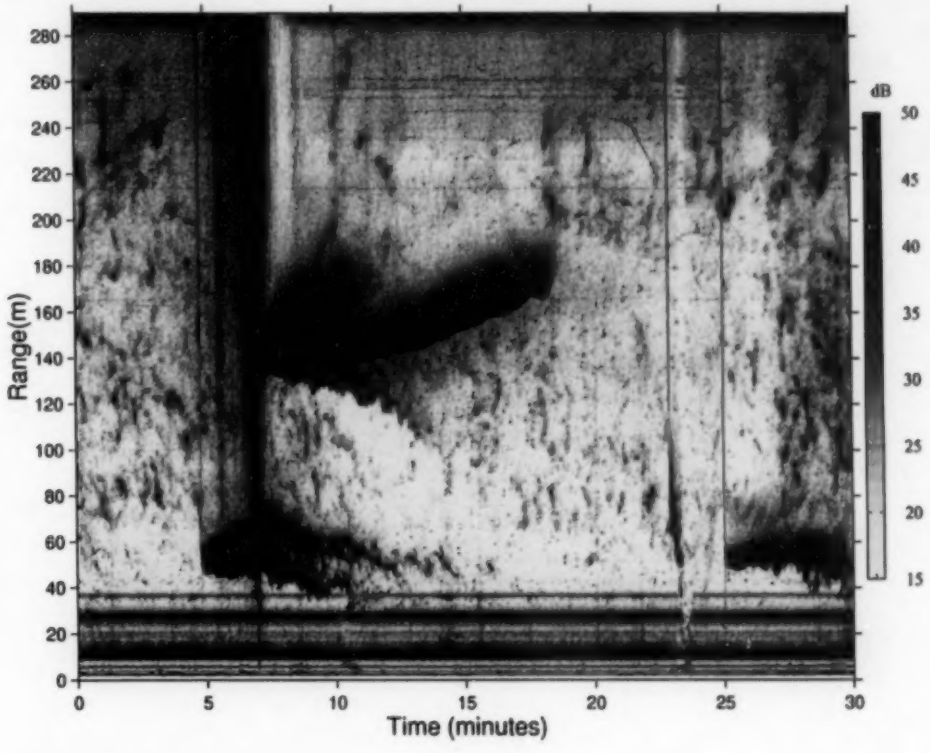
Fig. 6. Backscatter intensity (db re 1 digital count) echogram using the VH sidescan geometry starting 10:09:08 Sept. 20, 1995.



1–2 ms but generally decreased with range such that the multipaths could not be distinguished from the direct arrival beyond approximately 150 m range. Note that some of the multipaths (e.g., in Fig. 4 at 40–80 m range) revealed a slightly spread, more diffuse character presumably owing to reflections from a roughened sediment interface.

By assuming either a surface or bottom-reflected path, measurements of target range and multipath time delay were sufficient to calculate the fish depth, using simple trigonometry and $\phi_{incident} = \phi_{reflected}$ for specular scattering. At close ranges (<40 m) with the VV and VH sidescan geometry the large grazing angle surface reflections were resolvable, yielding fish depths near 4.5 m depth that were consistent with the depth of the narrow insonifying beam. At greater range the bottom reflections from fish near the surface (depth <5 m) were resolvable. By using a shorter transmit pulse and greater echo sampling rate, it would be possible to better resolve these multipaths. Note that the VV and HV sidescan geometries would be much less susceptible to multipath interference because of the narrow vertical acceptance angle.

Boat traffic and wind-induced surface roughness frequently interfered with fish detection. Figure 6 shows a VH mode echogram contaminated by boat traffic on the morning of September 20. The very intense object at 140 m range, persisting for roughly 12 min, was a log boom being towed downstream. The line of acoustic energy at all ranges coincident with the start of the log boom trace is probably due to cavitation-induced bubbles created by the tow boat propeller. The PSC fish counting boat, which constantly traversed back and forth across the river, created the two wake events near 55 m range. Also shown in Fig. 6 is a more diffuse, widespread scattering due to wind-induced surface roughness and bubbles (this image has not been processed to remove the background reverberation). At this time a roughly 8 m/s wind produced moderate choppy waves and some whitecapping on the river surface. This diffuse surface scattering was observed only at ranges >80 m (from this figure and others), implying that the vertically narrow sidescan beam intersected the water surface at that range. Clearly, any target counting and analysis scheme must be tailored to avoid such boat wake and surface roughness interference.

Quantitative analysis of backscatter levels

It is possible to make quantitative comparisons of measured background reverberation levels with both the acoustic models discussed above and the observed fish echo levels. The background reverberation levels were calculated by applying eq. 1, along with the calibration results, to the echo amplitude versus range averaged over many thousands of separate transmissions. Strong transient targets (e.g., fish and boat tracks) were removed from the background curves by an iterative ping-averaging process, which firstly calculated a simple time average at each range point, then repeated averaging with rejection of echoes more than 3 db above the base average. Figure 7 shows an example of these background reverberation curves for the horizontal fan-beam (VV) geometry. Comparisons between calm and windy (≈8 m/s) conditions showed a 25-db increase in the background reverberation at 100–150 m range,

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Fig. 7. Comparison of reference fish received level and modeled reverberation with experimental background reverberation versus range curves taken on Sept. 20 with VV sidescan geometry. Experimental curves averaged over 40 min, excluding fish targets. Model uses surface roughness and bottom scattering along with bubble layer scatter and absorption, calculated using surface values of $M_v = 3.4 \times 10^{-3}$ m^{-1} and $S_e = 2.9 \times 10^{-2}$ m^{-1}, with $L = 0.35$ m.

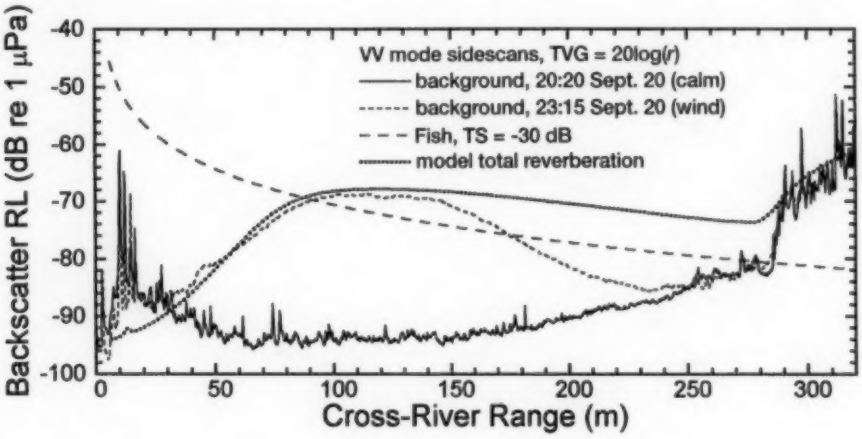
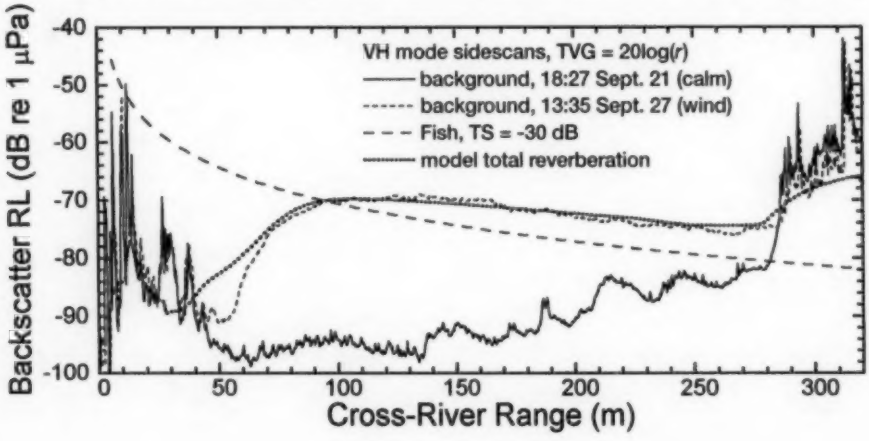


Fig. 8. Comparison of reference fish target strength and modeled reverberation with experimental background reverberation versus range curves taken on Sept. 21 and Sept. 27 with VH sidescan geometry. Reverberation model parameters same as in Fig. 7, except $L = 0.2$ m.



owing predominantly to bubble-layer scattering. The strong peaks in reverberation at close range (<20 m) were due to a combination of surface and bottom reflections through the sidelobes, reflections from fixed targets on the river bottom, and back-reflected echoes from the near river bank. These multiple surface and bottom reflections hindered fish track identification at close range. Log booms on the far bank were responsible for the strong echoes starting at 280 m.

Also shown in Fig. 7 are comparisons with reverberation and fish target strength models discussed earlier. The total reverberation is calculated using the semi-empirical models and constants outlined in section 2, along with the river cross section and transducer beam widths shown in Fig. 1 (necessary to calculate grazing angles and beam deviation losses). For the case with wind roughness and a bubble layer, the predicted and measured reverberation levels are in agreement (to ≈3 db) up to 150 m range. Modeled bottom scattering from the steep far river bank matches the increased measured RL beyond 280 m range. The strong decrease in measured reverberation (windy case) at ranges beyond 150 m was plausibly due to a decrease in bubble layer density and surface roughness on the far side of the river (at this time the wind direction was from the NNE, so that the north side of the river was shadowed and wind-wave activity increased towards the south bank). It is clear that for the case with wind-induced bubble layers, fish targets would not be observable beyond 70 m range. However, for the calm case, fish targets should be observable up to 250 m range.

Figure 8 shows a similar windy versus calm comparison of background reverberation levels for the VH (crossed) sidescan beam geometry. In this case the modeled and observed reverberation versus range curves were in direct agreement up to 280 m range. As with the VV sidescan geometry, it is clear

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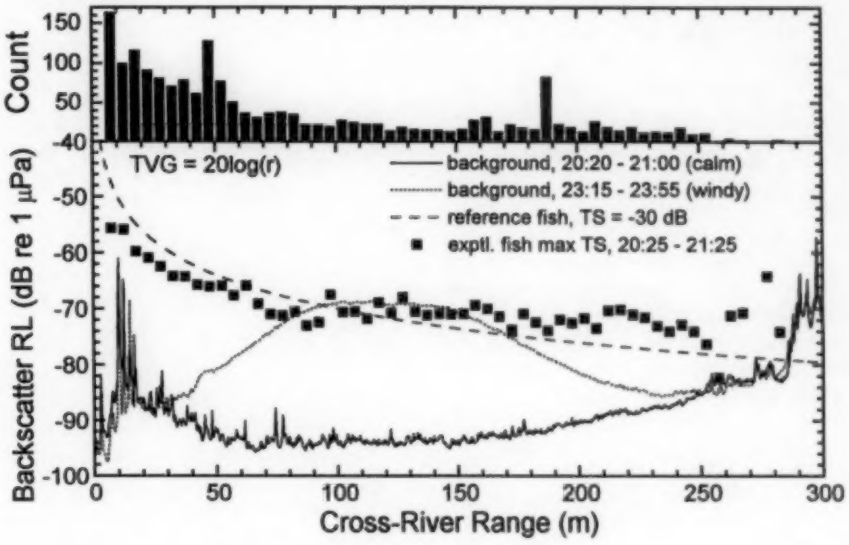
Fig. 9. Comparison of experimental fish target receive level with background reverberation curves taken on Sept. 20 with VV sidescan geometry. Fish target levels determined at closest approach of hyperbolic trajectory (near lateral incidence). Top panel shows number of targets averaged with 5-m range bins (1901 total targets).



that under calm conditions fish targets should be visible up to 250 m range, but under windy conditions, fish detection would be limited to <70 m.

Finally, Fig. 9 shows a direct comparison of these background reverberation versus range levels (windy and calm) for the VV sidescan geometry, with the observed echo levels from the measured fish trajectories. In this example, the fish tracks were visually recognized by the author over 1 h of data during the calm period, collecting roughly 1900 total fish tracks. For the VV sidescan hyperbolic trajectories, the maximum echo level occurred at the point of closest approach, coinciding with the lateral incidence TS maxima of the fish. The echo level of a TS = –30 db fish target is shown for reference. The observed fish echo strength decreased as 20 log(r) over the first 120 m as expected (remember the 20log(r) TVG). At close range the mean RL is less than the reference, owing to the inclusion of some targets on the edges of the sidescan beam where their effective TS would be smaller owing to beam deviation losses. Beyond roughly 120 m the measured fish RL increased relative to the reference owing to two effects: (*i*) with increasing range an increasing majority of fish now lay within the main beam lobe, and (*ii*) only the stronger fish echoes were recognized against the increasing background reverberation. Practically, it became very difficult to recognize fish tracks when the track-averaged RL (typically 10 db less than the maximum) fell below roughly 5 db above the background reverberation. This occurred under calm conditions at ranges greater than 200 m. Under windy conditions, it is again clear that surface reverberation would have prevented any fish track recognition beyond approximately 70 m range. The top panel of Fig. 9 also shows the observed cross-river target distribution (in 5-m bins), uncorrected for beam-pattern effects. Note that a small number of targets were detected at ranges in excess of 200 m. This distribution, considering the small insonified volume of this vertically narrow beam at close range, suggests that the fish target density greatly increased close to the near river bank.

Discussion

The study was aimed at assessing the feasibility for detection and counting of migrating salmon targets in the river, using sidescan sonar beams that partially insonified the river cross section and included backscatter from the river surface and bottom sediments. In this preliminary feasibility study, the sidescan system was able to identify clearly and quantify trajectories of migratory adult pink and sockeye salmon with typical length of 50 cm. Echo traces due to fish were readily observed in the first 70 m, and under ideal conditions traces were observed at ranges up to 250 m, limited by bottom reverberation near the far river bank. There was also some evidence of bias towards detecting only larger fish targets at range >120 m. One advantage of these sidescan sonars, especially in the wide vertical beam mode, was that a large proportion of the river cross section could be insonified in a single transmission. However, this incurred a penalty of interference from boundary reverberation and complications due to reflection multipaths. Such problems would also arise using dual or split-beam sonars in similar boundary grazing geometries. Unlike a split-beam sonar these sidescans could not provide direct estimates of fish TS, swimming direction, and vertical fish distribution. Still, useful stock assessment information can be extracted providing that appropriate counting techniques and corrections can be applied. For example, the swimming direction question can be resolved by pointing the sidescan beam either up or downstream by (say) 30°, introducing a distortion in the fish trajectories that reveals direction (Gaudet 1990). Unfortunately there are several other serious obstacles to be overcome before stock assessment using sidescans can be successful.

The cross-river fish target distribution is a key parameter

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for sonar performance and stock escapement estimates. Unfortunately, this calculation was often impossible owing to strong interference at range >60 m from boat wakes and wind-induced bubble layers. Moreover, even during calm periods with the absence of boat traffic, calculation of the true cross-river fish distribution would be problematic. Firstly, the insonified volume of the sidescan beam increased strongly and in a complicated manner with range. At close range (<10 m) the insonified volumes were less than 1 m^3, which was probably insufficient for effective target sampling, and near-field effects must be considered. At greater range (>100 m) the volume calculation was greatly complicated by the vertical limits imposed by the surface and bottom, and boundary reflection focusing effects. Even the simplest correction for insonified volume relative to the river depth at each range interval (as in Gaudet 1990) would require an assumption of the fish targets being evenly distributed in depth, which is highly doubtful. More sophisticated beam pattern compensation techniques based on echo statistics (e.g., Ehrenberg et al. 1984; MacLennan 1990) or echo counting (Kieser and Mulligan 1984; Thorne 1988) would similarly require an assumption of target densities being homogeneous transverse to the beam. Recent work by Mulligan and Kieser (1996) has adapted echo-counting models for nonuniform vertical fish distributions using a side-looking split-beam sonar, but this cannot be adapted to these simple beams where the target depth is not known precisely.

These surface-grazing beams were strongly susceptible to interference from both boat wakes and surface reverberation during windy periods. In many of the data sets, fish imaging was limited to the first 60–100 m range because of this interference. Tarbox and Thorne (1996) also observed degraded detection performance with a side-looking dual-beam system under windy conditions. Acoustic models of the total reverberation versus range, which included effects of bubble layer, boundary roughness, and volume scattering, were able to match experimentally measured background reverberation levels. Because of the potential for strong interference from surface reverberation, future deployments with side-looking narrow-beam sonars should include some kind of precision leveling or steering mechanism. Also, meteorological conditions should be monitored quantitatively.

Furthermore, acoustic multipaths from both surface and bottom reflections of the fish echo were observed. In some cases the time delay between the direct and reflected arrivals was sufficient to extract the target depth. Multipaths have the potential to confound automatic target tracking software by widening and disrupting the phase of the direct echo in the case of overlapping arrivals and by adding spurious targets for the case of separated arrivals. The amplitude and phase interference with direct arrivals has potentially serious implications for dual and split-beam sonar estimates of target strength and location. It is perhaps not generally appreciated that in this riverine geometry the magnitudes of these reflections will be comparable with the direct echoes (neglecting beam pattern effects). Specular acoustic reflections from the water surface will be nearly perfect at all angles, owing to the sharp contrast in sound speed and density between the two media. Specular reflection losses <2 dB can be expected from the calm to rippled surface of the Fraser River (Clay and Medwin 1977). In addition, the silty-sand bottom sediments will strongly reflect at all angles. To a first approximation this can be calculated from the sediment interfacial reflection coefficient (Clay and Medwin 1977):

$$(8)\qquad \mathbf{R} = \frac{\rho_2 c_2 \cos\phi_1 - \rho_1 c_1 \cos\phi_2}{\rho_2 c_2 \cos\phi_1 + \rho_1 c_1 \cos\phi_2}$$

in combination with Snell's Law, $\sin\phi_1/c_1 = \sin\phi_2/c_2$ (in these expressions ρ is bulk density, c is sound speed, ϕ is the incidence angle, and the subscripts 1 and 2 refer to water and sediments, respectively). For silty sands typical of the river bed, $c_2 = 1700$ m/s and $\rho_2 = 2020$ kg/m^3, so $\mathbf{R} \approx 0.4$ (–8 db) at near normal incidence. However, an important subtlety is that $\mathbf{R}$ approaches 1.0 above a critical incidence angle, i.e., at $\phi > \phi_{crit} = \arcsin(c_1/c_2) \approx 60°$ (i.e., at grazing angles <30°).

In this feasibility study, fish targets were identified visually, which was time consuming and obviously impractical for routine monitoring of potentially millions of migrating salmon. This user-interactive recognition scheme was necessary to allow rejection of interference from boat wakes, wind-induced bubble-layer scattering, multipathing, and non-salmonid targets. Computer-based target recognition schemes, which must be able to handle all these complications, should be developed and fine tuned if future, more-routine applications are pursued. It is speculated that neural-net pattern recognition algorithms could prove useful for this task. A further confounding factor was that fish targets were occasionally observed in high-density shoals, with passage rates estimated to be in excess of 5000 per hour. This has the potential to overload both manual and automatic target counting algorithms.

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Effects of the exotic zebra mussel (*Dreissena polymorpha*) on metal cycling in Lake Erie

Paul L. Klerks, Peter C. Fraleigh, and Jay E. Lawniczak

Abstract: This research demonstrated the impact of high densities of the zebra mussel (*Dreissena polymorpha*) on the cycling of copper, nickel, and zinc in a lake environment. Experiments with mussels on sedimentation traps in western Lake Erie and with mussels in flow-through tanks receiving Lake Erie water showed that zebra mussels remove metals from the water column, incorporate metals in their tissues, and deposit metals on the lake bottom. Removal of metals from the water column was estimated at 10–17%·day^{-1} of the amounts present. This material was largely deposited on the lake bottom; zebra mussels more than doubled the rate at which metals were being added to the lake bottom. Metal biodeposition rates were extremely high (e.g., 50 mg Zn·m^{-2}·day^{-1}) in high-turbidity areas with elevated metal levels. Two factors contributed to metal biodeposition by zebra mussels. First, their production of feces and pseudofeces increased the rate at which suspended matter was being added to the sediment (accounting for 92% of the increased metal biodeposition). Second, the material coming out of suspension had higher metal concentrations when zebra mussels were present (constituting 8% of the increased biodeposition).

Résumé : La présente recherche a démontré l'impact des densités élevées de moules zébrées (*Dreissena polymorpha*) dans le cycle du cuivre, du nickel et du zinc dans un environnement lacustre. Les expériences réalisées avec des moules dans des pièges de sédimentation dans l'ouest du lac Érié et avec des moules élevées dans des réservoirs à circulation recevant de l'eau du lac Érié ont révélé que les moules zébrées éliminent les métaux dans la colonne d'eau, incorporent les métaux dans leurs tissus et déposent des métaux dans le fond du lac. L'élimination des métaux dans la colonne d'eau a été estimée à 10–17 % de la quantité présente par jour. Ces métaux ont été en grande partie déposés sur le fond du lac; les moules zébrées ont plus que doublé la vitesse à laquelle les métaux sont ajoutés dans le fond du lac. Les taux de dépôt biologique des métaux étaient extrêmement élevés (p. ex. 50 mg Zn·m^{-2}·d^{-1}) dans les zones de forte turbidité présentant des concentrations de métaux élevées. Les moules zébrées ont contribué au dépôt biologique des métaux par deux mécanismes différents : premièrement, la production de matières fécales et de pseudofèces a augmenté la vitesse à laquelle la matière en suspension était ajoutée aux sédiments (expliquant 92 % de l'augmentation du dépôt biologique des métaux) et, deuxièmement, la matière qui cessait d'être en suspension présentait des concentrations de métaux plus élevées en présence des moules zébrées (comptant pour 8 % de l'augmentation du dépôt biologique).
[Traduit par la Rédaction]

Introduction

The presence of a biological component in the biogeochemical cycling of metals means that a change in community composition may affect the environmental distribution of metals. The introduction of the zebra mussel (*Dreissena polymorpha*) to the Laurentian Great Lakes in the mid-1980s (Hebert et al. 1989; Griffiths et al. 1991) has resulted in a substantial biotic change. This bivalve has become established at high densities in Lake Erie and other North American waters and does not appear to be replacing any similar and equally abundant species in its new environment. The presence of large densities of this suspension feeder may result in the entire volume of a lake passing through the zebra mussel population in a matter of days or weeks (Stanczykowska 1977; Reeders et al. 1989; Bunt et al. 1993). It is thus likely that the establishment of high densities of zebra mussels has modified metal cycling in North American waters.

Suspension-feeding bivalves may take up metals from the water column, where metals are present in physicochemical forms ranging from simple ions to colloids to metals sorbed on or incorporated in suspended particles. "Dissolved" metals are generally an important source of metals to suspension-feeding bivalves. Zebra mussels are capable of taking up cadmium, nickel, and zinc from the dissolved phase (Bias and Karbe 1985; Maarouf and Pihan 1987; Klerks and Fraleigh 1997). However, suspension-feeding bivalves also remove suspended material (including bacteria and algae) from the water column and ingest it, thus taking in metals associated with these particles. Uptake of particulate nickel and zinc by zebra mussels was shown to be a significant proportion of their metal accumulation and appeared dependent on particle abundance and composition (Klerks and Fraleigh 1997). Uptake of both dissolved and particulate metals may therefore be expected for zebra mussels. The high densities at which zebra mussels are found may translate into a significant accumulation of metals in zebra mussel populations.

Following uptake by zebra mussels, a proportion of metals is excreted as feces. The extent to which metals will be removed from ingested material appears to be element, substrate,

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and species dependent. Absorption efficiencies reported in the literature range from about 85% for *Macoma balthica*'s accumulation of copper and organo-selenium associated with algae (Luoma et al. 1992; Absil et al. 1994) to values below 30% for accumulation by *Mytilus edulis* of copper and cadmium associated with algae and for *Macoma balthica*'s removal of elemental selenium from sediments (Borchardt 1983; Riisgård et al. 1987; Luoma et al. 1992).

Zebra mussels may also remove particles (and associated metals) from the water column and not ingest this material but instead excrete it in the form of pseudofeces. The amount of material excreted as pseudofeces may be substantial, especially when the amount of suspended matter in the water column is high (Reeders and Bij de Vaate 1992). While material excreted as pseudofeces is initially returned to the water column, it will settle out fairly rapidly. Only about 8% of pseudofeces goes back into suspension (Walz 1978). Rates as high as 28 $mg \cdot cm^{-2} \cdot day^{-1}$ have been reported for sediment biodeposition by zebra mussels (Klerks et al. 1996). Even though zebra mussel pseudofeces are excreted through the inhalent siphon and feces are excreted through the exhalent siphon, it is very difficult to separate feces from pseudofeces after excretion. Because both are derived from the water column and end up together as biodeposited material, we have grouped metals in feces and pseudofeces for practical purposes.

This study investigated the effects of the zebra mussel on the cycling of three metals (copper, nickel, and zinc) in western Lake Erie by quantifying metal removal from the watercolumn, metal bioaccumulation, and metal biodeposition. Two approaches were used, because of the limitations inherent to each approach. Zebra mussels were kept in flow-through systems ("bioboxes") placed in a field station and continuously receiving Lake Erie water. In the other approach, mussels were placed at several locations in western Lake Erie using a sedimentation-trap design. Amounts of metal in various environmental compartments were quantified in these experiments.

Materials and methods

Biobox experiments

These experiments were conducted in bioboxes placed in the Toledo water intake. The location of this intake (in western Lake Erie) allowed the use of a flow-through design with the bioboxes receiving a continuous inflow (about 2 L/min) of Lake Erie water. The bioboxes (made of clear acrylic) had inside dimensions of 99.1 cm length, 22.0 cm width (thus a 2180 cm^2 bottom surface area), and 30.5 cm height. Four vertical baffles, situated alternately to the left and right side of the biobox, caused a serpentine flow of the water through the system. The baffles and a weir at the inflow to the biobox resulted in a division of the main part of the biobox into four equally sized compartments. The position of the outflow opening maintained water levels in the bioboxes at about 12.7 cm. Diagrams of these bioboxes have been published elsewhere (Klerks et al. 1993).

Two experiments were conducted during July–August 1993 and August–October 1993 (lasting 29 and 53 days, respectively). Mussels were placed in the bioboxes at four densities (0, 459, 917, and 1835 individuals/m^2 or 0–400 mussels per biobox) and were equally divided over the four main compartments in each biobox. Two replicate bioboxes were used for each density. Mussels used for the experiments were collected locally (inside or outside the water intake). The first experiment used a natural population of zebra mussels averaging 14.7 mm in length that had settled over a 9-month period on a substrate suspended in the water intake), while the second experiment used mussels that were 17.5–22.5 mm in length.

Concentrations of copper, nickel, and zinc were determined in the sediment accumulating in the bioboxes (no sediment was present at the start of the experiment). This sediment was sampled at the end of each experiment with the use of a piece of PVC pipe functioning as a small sediment corer, at random locations in each of the four main compartments of a biobox. Sediment samples were dried (24 h at 60°C), ground to a powder, and metals were leached from subsamples (about 1 g) in a 5-h extraction with concentrated nitric acid on a hotplate at 120°C. The resulting solution was filtered (using glass-fibre filters) and brought to volume with 0.4 M HNO_3. Metal concentrations were then quantified by flame atomic absorption (Perkin Elmer 1100B) or inductively coupled plasma spectrometry (Perkin Elmer Plasma II Emission Spectrometer). Extraction blanks and reference sediment (Buffalo River sediment, National Institute for Standards and Technology) were used for quality control. Our procedures (with $n = 13$) yielded concentrations of total extractable copper, nickel and zinc of respectively 89.3, 31.1, and 390 µg/g, compared with certified values for total metal levels (resulting from complete digestion of the sediment) of respectively 98.6, 44.1, and 438 µg/g in the standard reference material. Concentrations reported in this paper were not adjusted for the apparent incomplete extraction or incomplete recovery.

Total amounts of metals present in the sediment were calculated as the product of metal concentrations in the sediment and amount of sediment present. The latter data, and methodologies used to obtain these data, have been reported previously (Klerks et al. 1996). Values for metal concentrations in the sediment accumulating in the bioboxes and for metal mass deposition rates were combined for the two experiments. This was possible since there were no statistically significant interactions for these variables between experiment and effects under investigation. Where mean values of metal concentrations or deposition rates differed significantly between the two experiments, individual values were adjusted for these differences.

Tissue mass and tissue metal concentrations were determined in the zebra mussels at the start and end of the experiments. At the beginning of the experiment, mussels from the same batch and of the same size as used in the experiments were kept overnight in Lake Erie water to depurate. These mussels were then removed, blotted dry, and frozen (in 10 samples of 10 or 9 mussels each for the July–August experiment, 3 samples of 11 mussels each for the August–October experiment). At the end of the experiments, mussels were removed from the bioboxes and treated similar to the mussels used for determining initial tissue mass and metal levels. Metal analyses were done on 3 samples of 15 mussels for each biobox (July–August experiment) or 4 samples of 10 mussels for each biobox (August–October experiment). Soft tissues were later separated from the shells, dried (24 h at 60°C), and digested in 5 mL concentrated HNO_3 with 2 mL distilled water added. After refluxing for 4 h at 120°C, 2 mL of H_2O_2 (30%; Fisher certified grade) was added and heating was continued for an additional 2 h. After cooling, the tissue digest was filtered using glass-fibre filters and brought up to volume (25 mL) using 0.4 M HNO_3. Metal concentrations were then determined using flame atomic absorption for zinc and furnace atomic absorption for copper and nickel, using a Perkin Elmer 1100B. For quality control, blanks and reference material (oyster tissue, National Institute for Standards and Technology) were processed and analyzed with each mussel tissue batch. Our procedures (with $n = 9$) yielded concentrations of copper, nickel, and zinc in the oyster tissue averaging 62.4, 1.42, and 897 µg/g, respectively, compared with certified values for total metal levels of 66.3, 2.25, and 830 µg/g, respectively. For copper and zinc, the 95% confidence intervals around the mean overlapped with the uncertainty intervals listed for the oyster tissue reference material. In contrast, nickel levels were below the certified concentration. However, nickel concentrations in this material were close to our method detection limit and substantially lower than typical levels in zebra

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mussels. Values reported in this paper were not corrected for the possibly low nickel recovery. Mussel mortality averaged 5% during the July–August experiment and was less than 1% in the August–October experiment.

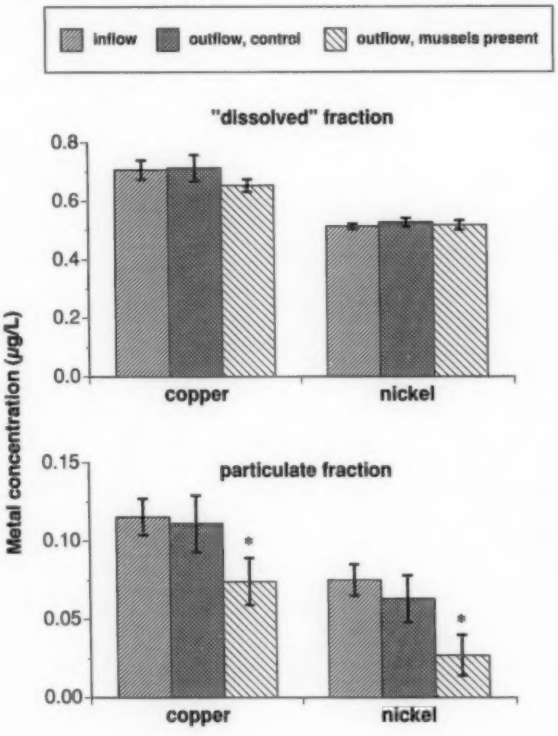
A 4-day biobox experiment was conducted during August 1995 to assess the effect of the zebra mussels on dissolved and particulate metal levels in this design (water samples collected during the earlier biobox experiments had been stored too long when satisfactory analytical procedures had been established). Mussels used for this experiment were collected from nearby Turtle Island and ranged in length from 17.5 to 22.5 mm. This experiment was conducted with two mussel densities (0 and 1835 individuals/m^2) and three replicate bioboxes per density. "Inflow" and "outflow" water samples were collected from sampling ports located on the bioboxes near the inflow and from the lines flowing out of the bioboxes. Samples were collected once daily on three consecutive days starting 1 day after the mussels had been placed in the bioboxes. Water temperatures during this period ranged from 26 to 26.5°C. Aliquots (500 mL) of each sample were filtered on acid-leached (overnight in 0.4 M HNO_3) 0.45 µm Millipore Triton-free HATF filters. Filters were subsequently digested during a 5-h heating at 120°C with trace-metal grade HNO_3. Water samples were acidified to pH <2 directly following the filtration. Metals in the filtered water were concentrated using the extraction procedure of Bruland et al. (1979). Minor modifications included the use of the chelators ammonium 1-pyrrolidinedithiocarbamate and diethylammonium diethyldithiocarbamate dissolved in water rather than in 1% ammonium hydroxide, a water sample volume of 350 rather than 250 mL, and a final volume of the extracted sample of 10 rather than 1.25 mL. Metal concentrations were quantified by atomic absorption using a Perkin Elmer 1100B spectrometer in flame (zinc) or graphite furnace (copper, nickel) mode. Recoveries for this method, obtained by concentrating spiked aliquots of the filtered water samples, were 99% (range 91–109%, $n = 4$), 77% (73–79%), and 101% (97–105%) for copper, nickel, and zinc, respectively. Concentrations reported here were not adjusted for low nickel recoveries.

Data from the biobox experiments were averaged for each biobox, and these means are the data reported here and subjected to statistical analyses. The biobox was thus the unit of replication. This averaging within each biobox was done to avoid pseudoreplication, as values obtained within the same biobox cannot be considered independent (e.g., mussels within a biobox receive the same water while filtration by mussels near the inflow of a biobox will result in reduced seston levels for mussels downstream in the same biobox).

Field experiments

These experiments used a sedimentation-trap design, consisting of glass funnels (funnel mouth diameter 12.20 cm) with a short piece of tygon tubing at the funnel stem connected to the mouth of a 40-mL glass vial. Plastic screening (with 7.5 × 7.5 mm openings) covered the funnel mouth, and supported the mussels enclosed in a mesh bag (with 3.3 × 2.7 mm openings). Mesh bags contained 20 mussels, resulting in a density of 1180 individuals/m^2 (at the funnel mouth). Mussels used in the first experiment (October 1994) were collected near West Sister Island and averaged 20.3 mm in length, while 17.5–22.5 mm mussels collected near Turtle Island were used for the second experiment (July 1995). Pairs of funnels (one with live mussels and a control one with empty pairs of shell valves glued together) were placed on a plastic-coated BOD rack. These racks were suspended in the west end of Lake Erie (Maumee Bay), with the use of an anchor (cement brick) and a subsurface float. In the first experiment, funnels were suspended approximately 1.5 m from the lake bottom at each of 12 sites in Maumee Bay for a period of 7 days during October 1994. In the second experiment, funnels were deployed for a period of 4 days during July 1995, suspended approximately 1.2 m from the lake bottom. This was done at 16 sites along a transect starting near the mouth of the Maumee River and extending to a distance of about 12 km into Maumee Bay. Methods were otherwise identical with the first field experiment. A more detailed description of the experimental set-up, materials, and methods has been provided elsewhere (Klerks et al. 1996). Metal concentrations in sediment accumulating in the funnels were determined following the same procedures as those used for the biobox experiments. Metal deposition rates were determined from these sediment metal concentrations and sediment deposition rates reported previously (Klerks et al. 1996). Metal biodeposition rates were determined for each pair of funnels as the metal deposition rates in the presence of live mussels minus the metal deposition rates in the presence of empty mussel shells.

Fig. 1. Concentrations of copper and nickel in Lake Erie water collected from inflow sampling ports ($n = 18$) and from outflow sampling ports of bioboxes without mussels (control; $n = 9$) and of bioboxes with a mussel density of 1835 individuals/m^2 ($n = 9$). Metal concentrations were determined in the "dissolved" fraction (<0.45 µm) and the particulate fraction. Asterisks denote a significantly ($p < 0.05$) lower concentration in the outflow than in the inflow in paired t-tests.



Results

Biobox experiments

In the 4-day experiment using this design, levels of particulate copper and nickel in the water exiting the mussel-containing bioboxes were significantly lower (in one-tailed paired t tests in which inflow and outflow data for each biobox were paired) than in the water entering each biobox (Fig. 1). These declines in particulate copper and nickel were 26 and 58%, respectively, of particulate metal levels in the water flowing into the bioboxes. No significant declines were observed for bioboxes without zebra mussels, nor for "dissolved" copper and nickel

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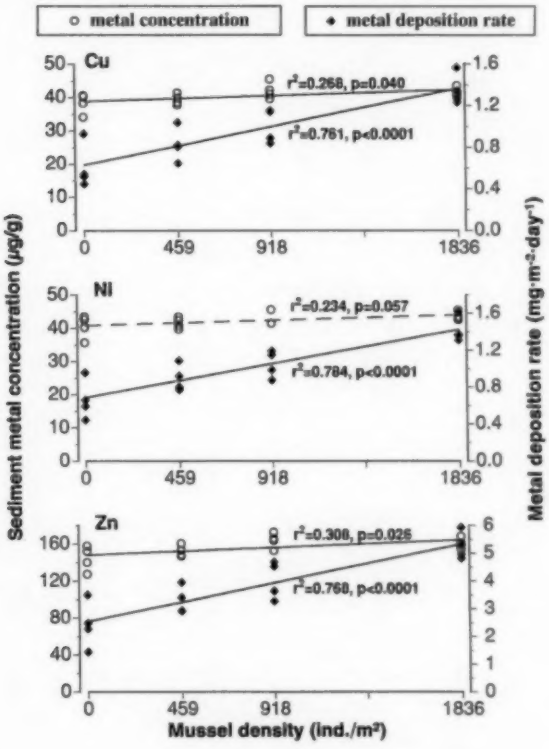
Fig. 2. Metal concentrations and rates of metal deposition for sediment accumulating in bioboxes receiving Lake Erie water as a function of mussel density. Biobox means and regressions shown. Results for July–August and August–October experiments combined.

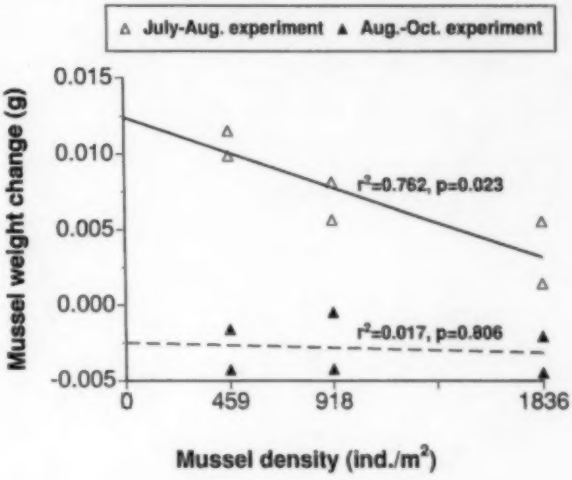


levels (Fig. 1). Zinc levels could not be determined accurately, because of background zinc levels in our laboratories that were relatively high in comparison to zinc levels in Lake Erie water.

In the July–August and August–October experiments, sediment accumulated on the bottom of the bioboxes at a rate that increased with mussel density. These rates have been published previously (Klerks et al. 1996). The concentrations of metals in this sediment also tended to exhibit a positive relationship with mussel density (Fig. 2). Significant regressions of metal concentrations on mussel density were found for copper and zinc but not for nickel. The combination of increases in the amount of sediment deposited and increases in metal concentration of this sediment with increasing mussel density, resulted in pronounced increases in the total mass of metal deposited. These increases were highly significant for all three metals (Fig. 2). Biodeposition by the zebra mussels was separated from normal deposition using the regressions of metal accumulation on mussel density, with normal deposition defined as the deposition at a mussel density of 0 (Fig. 2). At the highest density used in these experiments (1835 individuals/m^2), metal biodeposition roughly doubled normal deposition rates of copper, nickel, and zinc.

Changes in tissue biomass and in tissue metal concentrations were investigated, as both affect the amounts of metal present in the zebra mussel population. Tissue biomass

Fig. 3. Change in soft tissue dry weights during the July–August and August–Oct biobox experiments as a function of mussel density. Values are changes in average mussel weight for each biobox.



changed during the experiments, with an 18–56% increase (which was inversely related to mussel density) in the July–August experiment and a 13% decrease during the August–October experiment (Fig. 3). Concentrations of copper, nickel, and zinc in mussel soft tissue also changed during these biobox experiments (Fig. 4). Tissue levels increased for copper and nickel but decreased for zinc. These changes were statistically significant in the July–August biobox experiment. The same trends were observed in the August–October experiment, but changes were statistically significant for nickel only. The changes in tissue metal levels and in biomass resulted in net metal bioaccumulation rates that were substantially higher in the July–August experiment than in the August–October experiment (Fig. 5). Net bioaccumulation rates were generally positive, with the exception of zinc bioaccumulation during the later period. Copper and nickel bioaccumulation rates tended to be higher as mussel densities increased, while the net bioaccumulation rate for zinc tended to be negatively affected by mussel density (Fig. 5).

Field experiments

The specific design and especially the short duration of these experiments did not allow the assessment of metal bioaccumulation. However, sediment accumulation in the sedimentation traps was sufficiently high to allow an evaluation of sediment deposition rates and sediment metal levels. We reported previously that the sediment accumulation rate in funnels with live zebra mussels was 140% higher than in the corresponding control funnels (Klerks et al. 1996). In agreement with the biobox experiments, metal concentrations were slightly but consistently higher in the sediment deposited in the funnels with the live mussels compared with that in the control funnels (Fig. 6). This response was qualitatively similar among the three metals and the two experiments, though statistical significance (in the nonparametric Wilcoxon signed-rank test) varied somewhat. These two effects brought about by the

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Fig. 4. Concentrations of copper, nickel, and zinc in mussel tissues at the beginning and end of the July–August and August–October biobox experiments. Final concentrations at the three mussel densities were averaged (no significant treatment effects in ANOVA). Values are mean ± SE (n = 3–10, with a minimum of 9 mussels/sample). Asterisks denote statistically significant changes during the experiment in ANOVA (***, $p < 0.001$; **, $p < 0.01$).

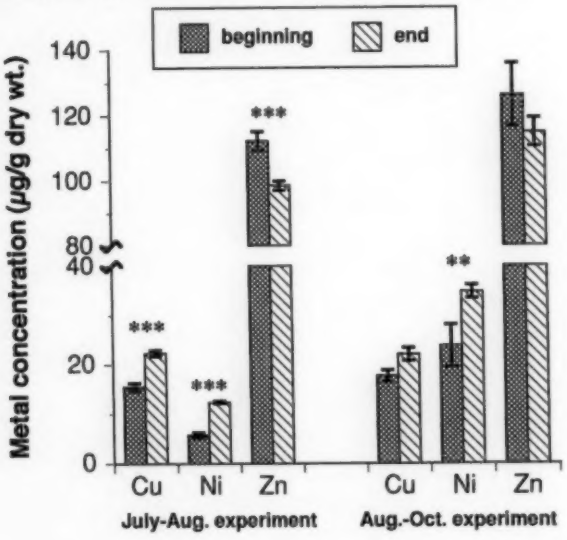
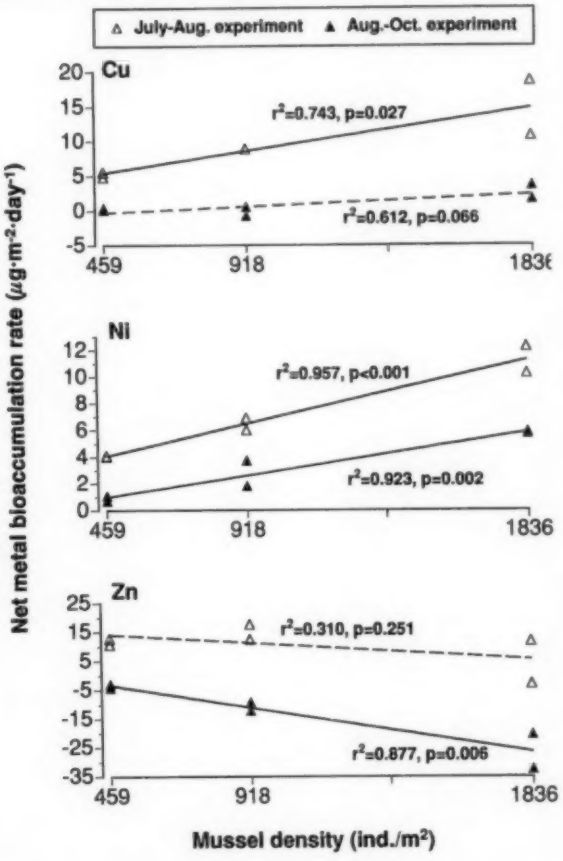


Fig. 5. Net metal bioaccumulation rates in soft tissues during the July–August and August–October experiments, as a function of mussel density. Biobox means and regressions are shown.



mussels (an increased sediment deposition rate and an increased metal concentration of this sediment) resulted in a large increase in the metal deposition rate. For all three metals and for both experiments, metal deposition rates were significantly higher for the funnels with live mussels than for the corresponding control funnels (Fig. 7). Biodeposition by zebra mussels (calculated as the increase in deposition in the presence of the live mussels) averaged over the three metals amounted to 35 and 412% of background metal deposition (deposition in the funnels with the dead mussels) in the October 1994 and the July 1995 experiments, respectively. Local conditions seemed to be important, since metal concentrations in sediment deposited in funnels with live mussels were significantly correlated to those of the corresponding control funnels (Table 1). One factor that proved to be important was the discharge of the Maumee River into Lake Erie. In the July 1995 experiment, concentrations of copper, nickel, and zinc in deposited sediment and metal biodeposition rates showed a dependence on the distance of the deployment site to the mouth of the Maumee River (Fig. 8). Very high metal biodeposition rates at sites close to the mouth of the river were due to somewhat elevated levels of metals in the seston combined with highly elevated sediment deposition rates. The latter was a consequence of very high levels of suspended matter, averaging 50 mg/L (Klerks et al. 1996).

Discussion

This research used two different approaches for determining the effects of zebra mussels on metal cycling. The biobox approach allowed a precise monitoring of many variables (including inputs and outputs, bioaccumulation, biodeposition). In spite of the use of natural lake water in a flow-through design it nevertheless constituted a somewhat unnatural setting. The field experiments using a sedimentation trap design constituted a more natural setting, but this approach was not well suited for determining variables other than biodeposition. We feel therefore that the two approaches can be considered complementary.

Within the two approaches used, experimental details differed between experiments. To some extent this resulted from experiments being designed to address different questions, e.g., a 4-day biobox experiment was sufficient for looking at changes in metal levels in water, whereas a longer time was needed to accurately measure biodeposition rates. Other changes were incorporated on the basis of obtained results (e.g., more sites were used in the second field experiments and sites were chosen to fall on a more distinct gradient in seston levels). Different experiments using the same approach should therefore not be considered replicated experiments and actually help to extend the validity of the results to more general conditions.

The zebra mussel densities used in our experiments (0–1835 individuals/m^2 for the biobox experiments and

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Fig. 6. Metal concentrations (mean ± SE, with $n = 12$ and $n = 16$, respectively, for the October 1994 and July 1995 experiments) in sediment deposited in funnels with live mussels and in control funnels (with empty shells). Asterisks denote a significant difference between the two groups in pairwise comparisons (nonparametric Wilcoxon signed-rank test; *, $p < 0.05$; **, $p < 0.01$).

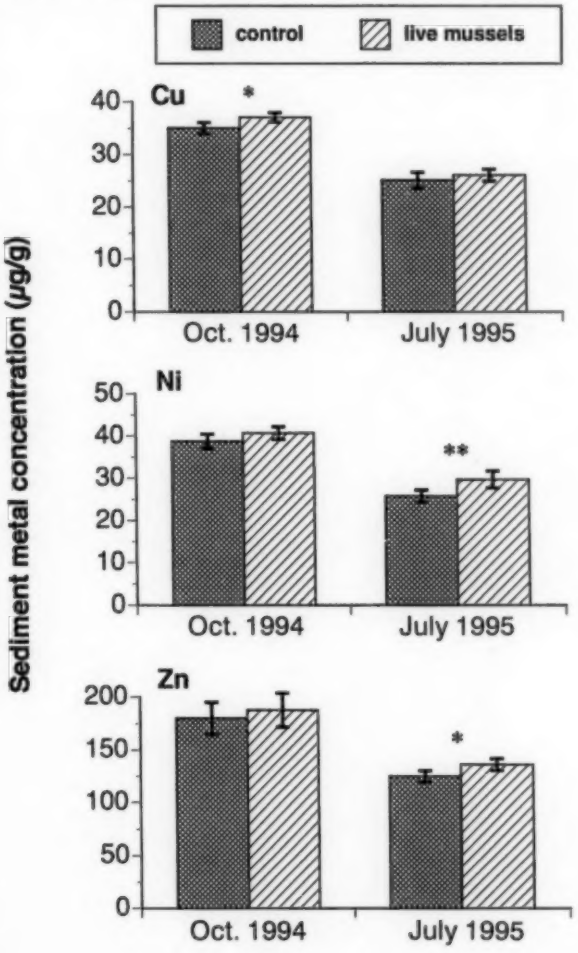
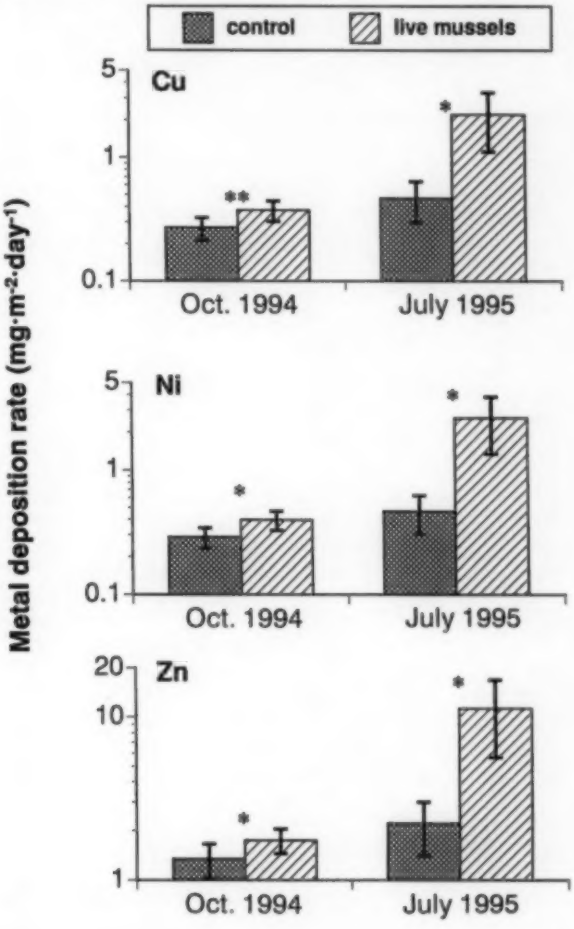


Fig. 7. Metal deposition rates (mean ± SE, with $n = 12$ and $n = 16$, respectively, for the October 1994 and July 1995 experiments) for funnels with live mussels and for control funnels (with empty shells). Asterisks denote a significant difference between the two groups in pairwise comparisons (nonparametric Wilcoxon signed-rank test; *, $p < 0.05$; **, $p < 0.01$).



1180 individuals/m^2 in the field experiments) are realistic for western Lake Erie. For example, sediment samples collected by us near Toledo in an area with soft sediment, contained clumps of zebra mussels ("druses") at a density of 16 500 individuals/m^2. Thirteen percent (or 2145 individuals/m^2) of these mussels were in the 17.5–22.5 mm range (with an additional 2% exceeding 22.5 mm). Substantially higher densities (as high as 341 000 individuals/m^2) have been reported for areas of western Lake Erie with hard substrates (MacIsaac et al. 1991; Leach 1993). Actual effects may thus be more pronounced than those reported here.

In our 4-day biobox experiment, levels of suspended copper and nickel were reduced by 26 and 58%, respectively, in measurements taken on 3 days. This compares with an average 49% decrease in turbidity at the same mussel density, measured on two occasions during the July–August and August–October biobox experiments when water turbidities were substantially higher (Klerks et al. 1996). Data reported in that study indicated that zebra mussels may remove 7–30% daily of the suspended matter in western Lake Erie. No reduction in dissolved metal levels was evident from the analyses of water flowing into and out of the bioboxes. However, uptake of dissolved metals may nevertheless have occurred. Static experiments with nickel and zinc radioisotopes (and thus very sensitive for detecting changes in metal levels) demonstrated that zebra mussels remove nickel and possibly zinc from the <0.45 µm fraction (Klerks and Fraleigh 1997). Support for the zebra mussel's ability to take up dissolved zinc is also provided by Maarouf and Pihan (1987). It is therefore apparent that the presence of zebra mussels will result in a substantial reduction of metal levels in the water column.

Since zebra mussels are suspension feeders, their reduction of water column metal levels is also supported by evidence of

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Table 1. Correlations (with corresponding p values) between metal concentrations in sediment deposited in funnels with live mussels and those in corresponding funnels with empty shells.

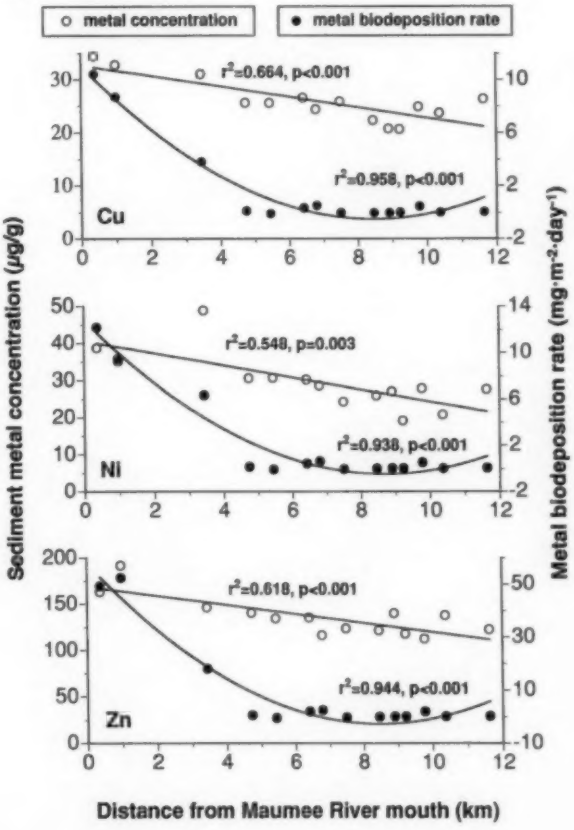
		r	p
Oct. 1994 experiment	Copper	0.596	0.039
	Nickel	0.755	0.003
	Zinc	0.520	0.084
July 1995 experiment	Copper	0.803	<0.001
	Nickel	0.733	0.002
	Zinc	0.644	0.011

their metal biodeposition and bioaccumulation. Based on estimates for metal bioaccumulation and metal biodeposition (see below), rates of removal of copper, nickel, and zinc from the water column of 0.6, 0.7, and 2.8 mg·m^{-2}·day^{-1}, respectively, are predicted for the summer–fall period. At an average depth of western Lake Erie of 7 m and total metal levels of 0.9 µg/L Cu, 0.6 µg/L Ni, and 4 µg/L Zn (this study), this amounts to a daily removal of 10, 17, and 10%, respectively, of the amounts of copper, nickel, and zinc present in the water column. There is no independent evidence yet for a decrease in water column metal levels following the introduction of the zebra mussel in Lake Erie or other lakes.

The presence of mussels has resulted in an increased rate of metal deposition on the lake bottom. This effect was observed in both of our experimental approaches. The mussels increased the rate at which metals were being deposited on the lake bottom by 35–400%. The increase in metal deposition as a function of mussel density was in part due to an increased sedimentation rate. We reported earlier that sediment biodeposition by zebra mussels can exceed normal sedimentation rates and that this biodeposition increases with increasing levels of suspended matter in the water column (Klerks et al. 1996). Zebra mussels also enhanced metal deposition via an increase in the concentrations of copper, nickel, and zinc in the deposited sediment. This effect was observed under field conditions as well as in the biobox experiments with flow-through Lake Erie water. The reason for the higher metal concentrations in the biodeposited sediment than in the normally deposited sediment is not directly evident but is in line with the observation that most metals tend to be present at higher levels in zebra mussel pseudofeces than in suspended matter (Reeders and Bij de Vaate 1992; Reeders et al. 1993). The relative contributions of changes in metal concentration and changes in sediment deposition rates to the increase in metal deposition rates were 8 and 92%, respectively. This result was consistent among the approaches used in this research: relative contributions of increases in metal concentration to increases in metal deposition were 7 and 9%, respectively, for the biobox and field experiments.

Concentrations of copper, nickel, and zinc in soft tissues of zebra mussels collected in western Lake Erie averaged 17, 15, and 119 µg/g dry weight. Zinc levels approached those in zebra mussel from relatively clean waters. Copper and nickel levels were generally above background levels, though not indicative of gross contamination by these metals (Busch et al. 1992; Mersch et al. 1992, 1996; Secor et al. 1993). Conclusions from this study may thus be valid for many other waters. The amounts of metal present in the zebra mussels were affected by changes in tissue metal concentrations (increases for copper and nickel, decreases for zinc) as well as changes in tissue biomass. The latter were shown to be dependent on mussel density (increases in tissue weight being less at higher mussel densities) and to vary between experiments (probably as a consequence of differences in water temperature and food availability). These results demonstrate that zebra mussels bioaccumulate substantial amounts of metals, in line with their extensive use in biomonitoring programs (Karbe et al. 1975; Léglize and Crochard 1987; Busch et al. 1992).

Fig. 8. Metal concentrations and metal biodeposition rates for sediment deposited in sedimentation funnels during the July 1995 field experiment, as a function of the distance of the site from the Maumee River mouth. Biodeposition determined as the difference in deposition at each site between the funnel with live mussels and the corresponding one with empty shells. Individual values and regressions are shown.



To compare the relative magnitudes of biodeposition and bioaccumulation, rates for these variables were calculated for our two biobox experiments (at an arbitrary density of 1000 individuals/m^2, because these rates were dependent on mussel density). This comparison (Table 2) shows that metal biodeposition was quantitatively much more important than bioaccumulation. However, this research did not provide true estimates of metal bioaccumulation and metal biodeposition by the zebra mussel population. For example, estimates were generally derived from a subset of the entire zebra mussel population (generally the 17.5–22.5 mm size-class) and did not incorporate recruitment of a new year-class.

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Table 2. Comparisons of net biodeposition and bioaccumulation rates at a density of 1000 individuals/m^2 in tanks ("bioboxes") receiving a continuous inflow of Lake Erie water.

		Net metal biodeposition rate ($\mu g \cdot m^{-2} \cdot day^{-1}$)	Net metal bioaccumulation rate ($\mu g \cdot m^{-2} \cdot day^{-1}$)
July–Aug. experiment	Copper	306	9.1
	Nickel	347	6.9
	Zinc	1400	23.1
Aug.–Oct. experiment	Copper	494	0.8
	Nickel	456	2.9
	Zinc	1636	–12.5

Note: Values were obtained from regressions of biodeposition and bioaccumulation rates on mussel densities (see Figs. 2 and 5).

A potentially more valid assessment of the relative importance of metal bioaccumulation and biodeposition can be obtained when results from other studies are included. For this assessment, the density of zebra mussels in western Lake Erie was estimated at 20 000 individuals/m^2 and the biomass at 200 g/m^2 shell-free dry weight. The latter was based on an average length of 10.7 mm (from a random sample of 100 mussels collected by us in western Lake Erie in July 1995), equivalent to a soft tissue dry weight of 10.0 mg/mussel on the basis of a reported relationship between mussel size and shell-free dry weight (Dermott et al. 1993). At a *P/B* ratio of 0.66 (Stanczykowska 1977), the annual soft tissue production amounts to 132 g/m2. At the average metal concentrations that we found for soft tissues of field-collected mussels (17 µg/g Cu, 15 µg/g Ni, and 119 µg/g Zn) and an estimated contribution of these metals in byssus threads and shells at 70% of the total (Van der Velde et al. 1992), amounts of metals incorporated by the mussels amount to approximately 7.5, 6.6, and 52 $mg \cdot m^{-2} \cdot year^{-1}$ for copper, nickel, and zinc, respectively. Amounts of metal deposited by the entire zebra mussel population can similarly be estimated. It appears that the individual size and population density of the adult mussels in our field experiments are a good representation of the age-2 cohort of the western Lake Erie zebra mussel population (Madenjian 1995). For the total zebra mussel population we used a multiplication factor of 2.56 (based on data from Madenjian (1995) for the contribution of the age-2 cohort to the total population's removal of algae). Seeing that our data were obtained during periods of relatively high mussel activity (July–October), we made the fairly conservative estimate that 120 days at these rates represent a full year's activity. These data resulted in biodeposition estimates of 186, 205, and 870 $mg \cdot m^{-2} \cdot year^{-1}$ for copper, nickel, and zinc, respectively. These estimates again point at the large effect of biodeposition by zebra mussels on the dynamics of metals relative to the actual metal bioaccumulation by the mussels. Of course, this does not mean that metal bioaccumulation is not important. Metal bioaccumulation may have pronounced ecological effects, as it could result in the transfer of metals up the food web. The latter has been demonstrated for cadmium accumulation in the tufted duck (*Anthya fuliga*) fed zebra mussels from a contaminated area (De Kock and Bowmer 1993).

In conclusion, this research points to a pronounced effect of the zebra mussel on metal dynamics, by removing copper, nickel, and zinc from the water column and depositing most of it on the lake bottom.

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Some properties of methane oxidation in a thermally stratified lake

Charles Bédard and Roger Knowles

Abstract: Profiles of $^{14}CH_4$ and ^{14}CO oxidation and concentrations of CH_4, NH_4^+, and nitrogen oxides (N_2O, NO_2^-, NO_3^-) were studied in Lake St. George, Ontario, during late summer stratification. Metalimnetic maxima of the nitrogen oxides occurred and high rates of oxidation of CH_4 and CO were limited to a narrow band (<1 m) below the nitrogen oxide peaks at the oxic–anoxic interface. Oxidation of CH_4 and CO was strongly inhibited by the nitrification inhibitor allylthiourea. The affinity of the oxidizers for CH_4 (approximate $K_m = 4.6$ μM), their sensitivity to picolinic acid, and the ratio of CH_4 oxidation to allylthiourea-sensitive CO oxidation together suggest that methanotrophs were responsible for the observed CH_4 oxidation. Pure cultures of *Nitrosomonas europaea*, although less sensitive to picolinic acid than the CH_4 oxidizers of Lake St. George, were affected by the inhibitor (approximate K_i for inhibition of NH_4^+ oxidation = 51 μM).

Résumé: Les profiles d'oxydation du $^{14}CH_4$ et du ^{14}CO, et de concentrations du CH_4, du NH_4^+ et d'oxydes d'azote (N_2O, NO_2^-, NO_3^-) ont été examinés dans le lac St-George, Ontario, durant la période de stratification estivale. Des maximums métalimnétiques d'oxydes d'azote étaient présents et les taux d'oxydation de CH_4 et de CO les plus élevés étaient limités à une étroite couche (<1 m) situées en-deçà de ces maximums, à la limite de la zone d'anoxie. L'oxydation du CH_4 et du CO était fortement inhibée par l'allylthiourée, un inhibiteur de la nitrification. L'affinité envers le CH_4 des microorganismes responsables de ces oxydations (K_m approximatif = 4.6 μM), leur degré élevé de susceptibilité à l'effet de l'acide picolinique, et le rapport oxydation du CH_4 sur oxydation du CO inhibée par l'allylthiourée, ensemble, suggèrent la prédominance des bactéries méthanotrophes. Les cultures axènes de *Nitrosomonas europaea*, bien que moins susceptibles à l'effet de l'acide picolinique que les microorganismes du lac St-George responsables de l'oxydation du CH_4, sont quand même affectées par cet inhibiteur (K_i approximatif de l'oxydation du NH_4^+ = 51 μM).

Introduction

Peaks of nitrogen oxides near the boundary between oxygenated and anoxic waters are a common feature of stratified lakes (Hutchinson 1957); associated with and just below such peaks is often a maximum of CH_4 oxidation leading to the suggestion that methanotrophs may be responsible for accumulation of NO_2^- observed at the same depth (Hanson 1980; Harrits and Hanson 1980). The relative roles of methanotrophs and nitrifiers in such habitats is the subject of some speculation (reviewed in Bédard and Knowles 1989; Hanson and Hanson 1996). Hence, we studied, in Lake St. George, Ontario, the stratification of nitrogen oxides and CH_4, and oxidation of CH_4 and CO, both of which activities can be carried out by both methanotrophs and ammonia-oxidizing bacteria (Bédard and Knowles 1989). To determine which group of bacteria was responsible for CH_4 oxidation in the water column, we also determined the kinetics of CH_4 oxidation and the sensitivity of CH_4 and CO oxidation to allylthiourea and picolinic acid which are reported to exert somewhat differential effects on methanotrophs and ammonia oxidizers (Bédard and Knowles 1989; Megraw and Knowles 1990).

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Methods

Study site and sampling

Lake St. George (43°57′30″N, 79°25′30″W) is a mesotrophic, double basin, kettle lake (10.3 ha) located 2 km west of Oak Ridges, Ontario (Knowles and Lean 1987; Knowles et al. 1990; Bédard and Knowles 1991). All samples were taken from a station situated above the deepest part (15.3 m) of the eastern basin (5.88 ha). Water samples were obtained through tygon tubing connected to a peristaltic pump, and the metalimnion was sampled at narrower depth intervals than was the rest of the water column because high rates of CH_4 and CO oxidation were mostly restricted to this zone. Temperature and oxygen concentrations were measured with a Yellow Springs Instruments model 54A probe. Oxygen data below approximately 5 μM are probably unreliable owing to sulfide interference, H_2S being detectable by smell in samples from the hypolimnion. Water samples for gas analyses were obtained in 350-mL bottles. Three volumes were allowed to overflow before addition of 2 mL of 5 M NaOH. Bottles were immediately capped, excluding, as far as possible, any air bubbles. Gases were extracted by multiple phase equilibration and analyzed by gas chromatography (GC, see below).

Analyses and assays

Nitrous oxide and CH_4 were measured using electron capture and flame ionization detectors, respectively (Chan and Knowles 1979; Knowles et al. 1981). In 1984, dissolved inorganic nitrogen (DIN) analyses were carried out by the Canada Centre for Inland Waters (Burlington, Ont.) as described in Knowles et al. (1981). Samples for DIN analyses in 1985 were taken in acid-washed, plastic scintillation vials. Ammonium, NO_2^-, and NO_3^- were measured on an autoanalyzer

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(Chemlab Instruments, Hornchurch, England); NH_4^+, by the indophenol-blue method, and NO_3^-, following reduction with hydrazine–copper, and NO_2^-, by the Griess reaction.

Rates of CH_4 and CO oxidation in water samples were determined using $^{14}CH_4$ (specific activity of 2.18 Gbq·mmol^{-1}; Amersham) and ^{14}CO (0.37 Gbq·mmol^{-1}; ICN). Radiolabelled gases were diluted with N_2 and stored at room temperature and atmospheric pressure in either serum bottles filled with an appropriate amount of distilled water and stoppered with silicone rubber-reinforced SubaSeals or in mercury manometers. In the serum bottles, correct pressure was maintained by injecting a volume of distilled water equal to the volume of gas to be removed. Methane and CO concentrations in the containers were measured using GC immediately before departure to the field. Methane was determined as above and CO was analyzed on a Perkin Elmer model 3920 GC equipped with a 174 cm × 3.3 mm o.d. Molecular Sieve 5A column and He carrier with a thermal conductivity detector.

In 1984, CH_4 and CO oxidation were measured at in situ O_2 concentrations using a method modified from Rudd et al. (1974). Duplicate 60-mL serum bottles were filled with water from each depth, allowed to overflow several volumes and then, excluding any headspace, capped with grey butyl rubber stoppers and aluminum crimps. They were kept cool and assays started within 4 h of sampling. Using a disposable plastic syringe with Mininert valve or a Hamilton gas-lock syringe, 0.5 mL of the mixture of $^{14}CH_4$ and N_2 containing 64.3 nmol (0.14 MBq) of $^{14}CH_4$ were injected into each assay bottle and displaced water collected in another syringe such that the final headspace volume was 0.5 mL. For ^{14}CO, 91.3 nmol were injected in a total volume of 0.25 mL. The bottles were vigorously shaken by hand for 1 min. Controls received 200 µL of 4 M NaOH immediately before the addition of label. The bottles were placed in onion bags, suspended on a nylon rope, and incubated for 17 h at the depth from which they were collected. After incubation, 200 µL of 4 M NaOH were injected into each bottle to raise the pH and arrest activity. Samples were shaken and allowed to stand for 1 h to ensure absorption of $^{14}CO_2$ into solution, after which 4 mL were transferred to a scintillation vial. The vial was left uncapped for several hours to allow escape of any $^{14}CH_4$ or ^{14}CO remaining, and 14 mL of scintillation cocktail (Aquasol) were then added. Counting was done in a Beckman model LS 7500 liquid scintillation counter with an efficiency of 82% (channels ratio method). Because lake-water CH_4 concentration data were available, in situ rates of oxidation could be calculated.

In 1985, CH_4 and CO oxidation were measured by a method based on that of Jones et al. (1984) except that allylthiourea (readily soluble in water) instead of nitrapyrin was used to determine inhibitor-sensitive CO oxidation. Jones et al. (1984) proposed that, to distinguish between methanotroph-dominated and nitrifier-dominated aquatic systems, the ratio of CH_4 oxidation to inhibitor-sensitive CO oxidation should be used. At CH_4 and CO concentrations of 11.9 and 2.2 nM, respectively, Jones et al. (1984) observed ratios of 0.0007 to 0.0428 for ammonia oxidizers and 0.380 to 1.87 for methanotrophs. In the 1985 study, water samples in polyethylene bottles were kept cool, then sparged with air for 10 min using an aquarium pump to remove dissolved CH_4 and CO. They were thus at 100% air saturation. Five millilitres of sample were then dispensed into a 15-mL serum bottle, which was capped and crimped as before. Radiolabelled gas was then injected by syringe, giving final concentrations of 85.4 nM CH_4 and 84.6 nM CO (based on Ostwald coefficients; Wilhelm et al. 1977). Samples were incubated at 10°C on a rotary shaker at 100 rpm for 12 h except where otherwise indicated. Activity was arrested by addition of 200 µL of 5 M NaOH, and samples were processed as for the 1984 study except that 2 mL of sample and 6 mL of scintillation fluid were added to scintillation vials. For determination of K_m (concentration giving half maximum activity) and V_{max} (maximum activity) of CH_4 oxidation, all samples received the above-indicated amounts of $^{14}CH_4$ and varying amounts of unlabelled CH_4 (Canlab) as required. In the inhibitor experiments, aqueous solutions of allylthiourea (98%; Aldrich) and picolinic acid (99%; Aldrich) were added in 100-µL aliquots. The final concentration of allylthiourea was 86 µM (10 mg·L^{-1}).

In 1984, some measurements of nitrapyrin-sensitive dark ^{14}C-bicarbonate uptake (Billen 1976) at in situ O_2 concentrations were made. Nitrapyrin (>99%; gift from Dow Chemical Co.) dissolved in dimethyl sulfoxide (DMSO), was added to duplicate 60-mL serum bottles to give a final concentration of 43 µM. Controls received DMSO only. Final DMSO concentration was 100 µL·L^{-1}. Final concentration of ^{14}C-bicarbonate (2.07 Gbq·mmol^{-1}; ICN) was 9.2 kBq·mL^{-1}. All bottles were incubated in the dark, in situ, as described above. Activity was arrested by filtering through a 0.45-µm Millipore HA filter, which was then placed in a scintillation vial and covered with 1 mL of 0.5 M HCl. After ca. 3 h, 9 mL of Aquasol were added and samples were counted as described above.

Also in 1984, some measurements of H_2 oxidation using H^3H at in situ O_2 concentrations were made by adding 1 mL of H^3H (specific activity 7.3 Mbq·mL^{-1}; Matheson) to sealed 60-mL serum bottles prepared and processed as for $^{14}CH_4$ and ^{14}CO assays (above).

Bacterial counts

Samples for microscopy were preserved with 2% v/v glutaraldehyde. Enumeration of bacteria and cyanobacteria was done by epifluorescence microscopy (D. Bird, Université du Québec à Montréal, Montréal, QC H3C 3P8, Canada; personal communication). Preserved sample (2 mL) was placed in a Millipore filter funnel and DAPI (diamidino-2-phenylindole) was added to a final concentration of 0.5 µg·mL^{-1}. After 10 min, the sample was gently (10 cm Hg vacuum) filtered through a 0.45-µm Nuclepore filter stained with Irgalan black. The filter was layed on a microscope slide, cleared with non-fluorescent immersion oil, and covered with a cover glass. The Zeiss microscope possessed a G365 excitation filter, an FT395 dichroic mirror, and an LP420 barrier filter. Cyanobacteria were enumerated by autofluorescence using a BP450–490 excitation filter. At least 400 each of cyanobacteria and nonpigmented bacteria were counted.

Pure culture study

Nitrosomonas europaea (from E.L. Schmidt, University of Minnesota, St. Paul, MN 55108, U.S.A.) was grown and tested for contamination as described by Hynes and Knowles (1982). Several 1-L batch cultures were harvested by three times centrifugation from and resuspension in N-free growth medium buffered with 0.1 mM HEPES. The final protein concentration was 108 µg·mL^{-1} as determined by the Biuret method (Gornall et al. 1949) because HEPES interfered with Lowry determinations. Bovine serum albumin was used as the standard. For the assay of NH_4^+ oxidation, 1.9 mL of cell suspension in a 15-mL serum bottle was supplemented with 100 µL of a solution containing NH_4Cl (final 10 mM) and, when required, picolinic acid in N-free HEPES-buffered medium adjusted to pH 7.5. Bottles were capped as above and incubated at 30°C on a rotary shaker at 240 rpm. Samples (0.25 mL) of headspace were removed by syringe and C_2H_2 was injected to a concentration of 1 kPa to stop NH_4^+ oxidation (Hynes and Knowles 1982). Nitrous oxide was determined as described above and O_2 was detected on the same GC used for the CO analysis described above. Culture samples (2 mL) were frozen until analysis of NO_2^- and NH_4^+ as outlined above. All experiments were carried out within 3 days of harvesting, and no change in activity was noted over this period.

Results

Depth profiles

Peaks of nitrogen oxides as well as CH_4 and CO oxidation tracked the oxic–anoxic interface in both 1984 and 1985 (Figs. 1 and 2), and O_2 fell below detectable levels at or within 0.5 m of the depth of maximum CH_4 oxidation. In 1984 the interface was located 2 m shallower than in 1985. Oxygen

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Fig. 1. Profiles of temperature, concentrations, and activities on August 30, 1984.

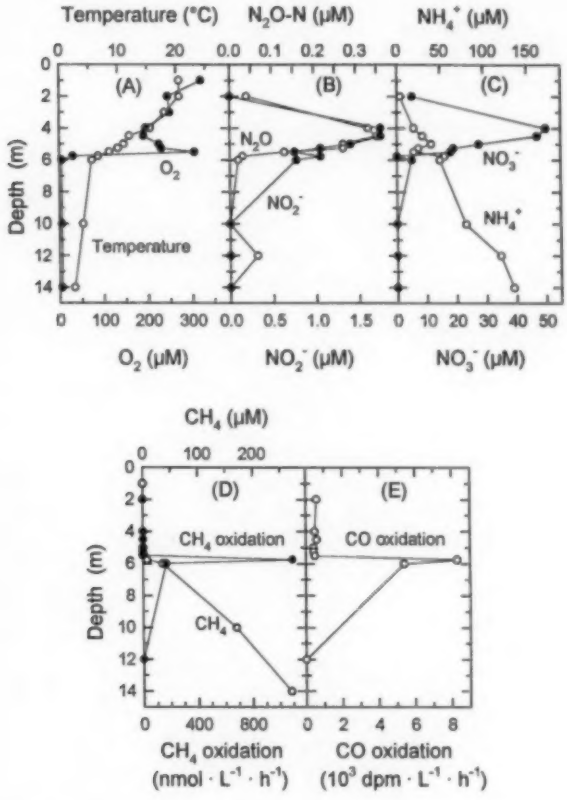
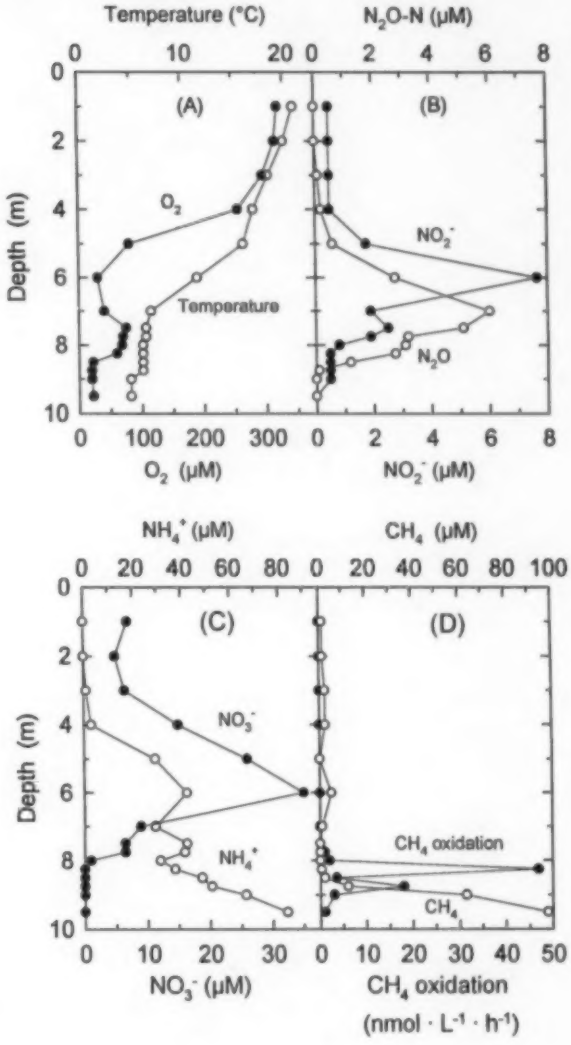


Fig. 2. Profiles of temperature, concentrations, and CH_4 oxidation on September 3, 1985.



demand in the tropholytic zone of lakes is determined in a general way by the level of primary productivity (Hutchinson 1957), and this was apparently higher in Lake St. George in 1984 than in 1985. Secchi disk depths were consistent with this conclusion because from June 4 to August 27, 1984, they were 1.5 ± 0.3 m (mean ± SEM), but from June 3 to September 2, 1985, they were 3.2 ± 0.2 m (D.J. McQueen, York University, Downsview, ON M3J 1P3, Canada; unpublished data). This would have made the lake eutrophic in 1984 and mesotrophic in 1985 (Forsberg and Ryding 1980).

Peaks in O_2 were seen immediately above CH_4 oxidation peaks in both years. The very high O_2 value noted in 1984 (Fig. 1) was a persistent feature of the 5-m depth in that year and was present from August 13 to September 10 with O_2 concentrations of 190–500 μM being observed (Knowles et al. 1990). The peak was probably related to a large population of cyanobacteria and photosynthetic flagellates noted in these depths (Fig. 3) in both years.

There was clear spatial separation between the peaks of nitrogen oxides and those of CH_4 and CO oxidation. The former were found 1.25–2.25 m above the latter. In 1984, in situ rates of CH_4 oxidation measured at 5.75 and 6 m were 25–200 times greater than elsewhere in the water column. There was no measurable activity in the hypolimnion. The mean rate at depths above 5.75 m, where the average CH_4 concentration was 2.03 μM, was 3.00 ± 0.75 nmol·L^{-1}·h^{-1}. This represents a turnover time of 28 days, compared with a turnover time of 8 h at 5.765 m where CH_4 was 8 μM.

Despite the use of a different method, the pattern of oxidation that emerged in 1985 (Fig. 2) was qualitatively similar to that seen in 1984. In 1985, assay conditions were such that O_2 concentrations were saturating at the beginning of the incubation. If O_2 had a deleterious effect, this was not readily apparent because CH_4 oxidation rates as high as or higher than in 1984 were measured in the kinetics experiment described below. The mean CH_4 oxidation rate at 8 m and above was 0.30 ± 0.08 nmol·L^{-1}·h^{-1}, more than two orders of magnitude lower than the highest rate noted at 8.25 m.

The highest CO oxidation rates occurred at the CH_4 oxidation peak in both 1984 (Fig. 1) and 1985 (Table 1), and there was never any indication of increased oxidation of CH_4 or CO

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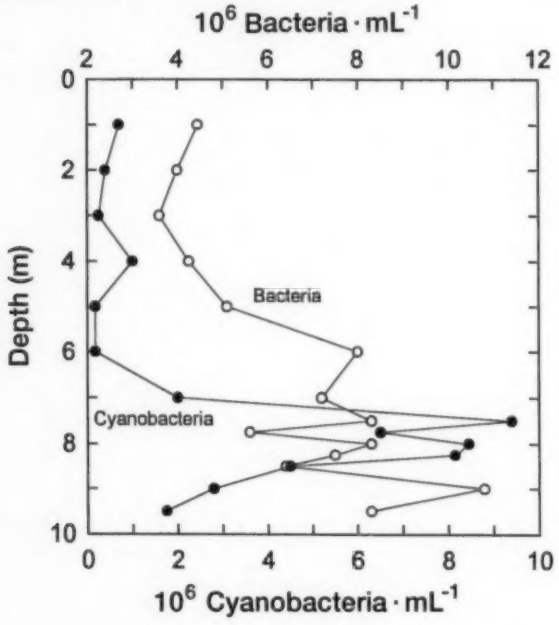

Fig. 3. Profiles of bacterial (○) and cyanobacterial (●) numbers on September 3, 1985.

at the depths of the NO_3^- maximum in either year. In 1984, some measurements of nitrapyrin-sensitive $H^{14}CO_3^-$ uptake and $^3H_2$ oxidation were made (Table 2). The highest rates of both activities were found at the depths of maximum CH_4 and CO oxidation. Some methanotrophs possess hydrogenase (Chen and Yoch 1987) as well as nitrogenase-dependent H_2 uptake (de Bont 1976) and, therefore, may be responsible, at least in part, for the high value of H_2 uptake noted at 6 m.

Bacterial densities in the metalimnion and upper hypolimnion were nearly twice as high as in the epilimnion in 1985 (Fig. 3). A cyanobacterial peak occurred between 7 and 9.5 m and appeared to consist mostly of a single-celled, coccoid form. Numerous dividing cells were present, suggesting that considerable growth was occurring.

Kinetics and effect of inhibitors

The kinetics of CH_4 oxidation in an 8.25-m water sample obtained on September 3, 1995, are shown on an Eadie–Hofstee plot (Fig. 4). The apparent K_m and V_{max} values, estimated by regression analysis, were 4.6 μM and 3.1 μmol·L^{-1}·h^{-1}, respectively.

Cell suspensions of *Nitrosomonas europaea* produced NO_2^-– from NH_4^+ at a rate of 0.89 ± 0.01 mmol·L^{-1}·h^{-1}, and N_2O production represented 0.42% of the NO_2^- formed. Picolinic acid inhibited NH_4+ oxidation by such suspensions with a K_i (concentration giving 50% inhibition), estimated by regression analysis, of 51 μM (Fig. 5). The pattern of inhibition was essentially the same for N_2O formation.

Picolinic acid inhibition curves for CH_4 oxidation at three different depths in Lake St. George on August 31, 1985, are superimposed on the inhibition curve for NH_4^+ oxidation by *Nitrosomonas* (Fig. 5; rates of CH_4 oxidation at the various depths are listed in Table 1). Picolinic acid at a concentration of 5 μM inhibited CH_4 oxidation by an average of 53 ± 3% suggesting a K_i value at or near this concentration. This is one order of magnitude lower than the K_i for *Nitrosomonas*. It was not possible to estimate the K_i for CH_4 oxidation because there were no data for concentrations of picolinic acid giving less than 45% inhibition.

The effect of picolinic acid and allylthiourea on CO oxidation as well as the ratio of CH_4 oxidation to allylthiourea-sensitive CO oxidation were measured (Table 1). Allylthiourea inhibition data suggest that either methanotrophs or ammonia oxidizers were responsible for most of the CO oxidation measured at the depths of maximum CH_4 oxidation. In this zone, allylthiourea inhibited CO oxidation by 77.5–100%. On the one occasion when significant CO oxidation was found outside the zone (September 3 at 2 m), the organisms responsible were apparently neither methanotrophs nor ammonia oxidizers because they were insensitive to both allylthiourea and picolinic acid.

At the depths of maximum CH_4 oxidation, 40 μM picolinic acid inhibited CO oxidation by 34.6–100%. The possible significance of this inhibition is examined in the Discussion. Ratios of CH_4 oxidation to allylthiourea-sensitive CO oxidation fell between 0.14 and 1.6, suggesting that it may be a methanotroph-dominated system (Jones et al. 1984).

Discussion

The summer profiles of CH_4 oxidation in Lake St. George are qualitatively similar to those in Lake 227 of the Experimental Lakes Area, Ontario, and Lake Mendota, Wisconsin (Rudd and Hamilton 1975; Harrits and Hanson 1980). In all three bodies of water, significant rates of CH_4 oxidation are limited to a narrow lens of water in the thermocline where O_2 concentrations often fall below the limit of detection of temperature–oxygen probes (ca. 5 μM) and do not generally exceed 30 μM. Within this lens, maximum CH_4 oxidation rates are in the order of 1 μmol·L^{-1}·h^{-1} (Rudd et al. 1974; Harrits and Hanson 1980). The width of the layer of high activity in Lake St. George and Lake 227 is approximately 0.5 m, but in Lake Mendota, it varies between 4 and 6 m (Rudd and Hamilton 1975; Harrits and Hanson 1980). These values are thought to relate directly to rates of vertical diffusion in the thermocline (Fallon et al. 1980). Lake Mendota is by far the largest of the three lakes and is more subject to wind mixing, which reduces the stability of the water column. Diffusion coefficients within water columns are inversely proportional to their stability (Quay et al. 1980). The lack of O_2 in the hypolimnion precludes CH_4 oxidation there. Low CH_4 concentrations (<1 μM) are believed to limit rates of oxidation in the epilimnion of Lake Mendota (Harrits and Hanson 1980). Methane levels in the epilimnion of Lake 227 rose as high as 5 μM, but in the vicinity of the CH_4 oxidation peak, concentrations frequently dropped below 0.1 μM (Rudd and Hamilton 1975). A similar pattern was noted in Lake St. George. In the Lake 227 epilimnion, CH_4 oxidation was limited by an apparent inorganic nitrogen (DIN) limitation that resulted in a sensitivity to >30 μM O_2 of the putative dinitrogen-fixing cells (Rudd et al. 1976). The CH_4 oxidizers of Lake St. George showed no particular sensitivity to O_2 suggesting that they do not need to fix N_2. DIN values here are, in general, considerably higher than

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Table 1. Inhibition of CO oxidation by picolinic acid and allylthiourea, and CH_4 to CO oxidation ratios in water samples from Lake St. George.

		CO oxidation[a]				
Date	Depth (m)	Rate (nmol·L^{-1}·h^{-1})	Inhibition by 40 µM picolinic acid (%)	Inhibition by 85 µM allylthiourea (%)	CH_4 oxidation[a] (nmol·L^{-1}·h^{-1})	Ratio of CH_4 oxidation to allylthiourea-sensitive CO oxidation
Aug. 31, 1985[b]	8.00	18.1	82.4	100	3.57	0.20
	8.25	23.2	89.2	85.1	2.69	0.14
	8.50	19.8	100	100	8.94	0.45
Sept. 3, 1985[c]	2.00	11.3	10.4	2.4	0.12	*d*
	8.25	35.6	34.6	82.2	48.0	1.60
	8.50	4.7	42.2	77.5	3.95	1.10
	8.75	21.5	76.5	100	18.4	0.90

[a]All values are based on duplicate samples.
[b]6-hour incubation.
[c]12-hour incubation.
[d]CO oxidation was not sensitive to allylthiourea.

Table 2. Nitrapyrin-sensitive ^{14}C-bicarbonate uptake and $^3H_2$ oxidation at different depths on August 30, 1984.

Depth (m)	Nitrapyrin-sensitive ^{14}C–HCO_3^- uptake (µmol C·L^{-1}·h^{-1})	$^3H_2$ oxidation (dpm·L^{-1}·h^{-1})
2.00	0	—
5.25	11.67	—
5.50	11.67	0
5.75	25.00	—
6.00	28.33	4507
10.00	—	284
14.00	*	1345

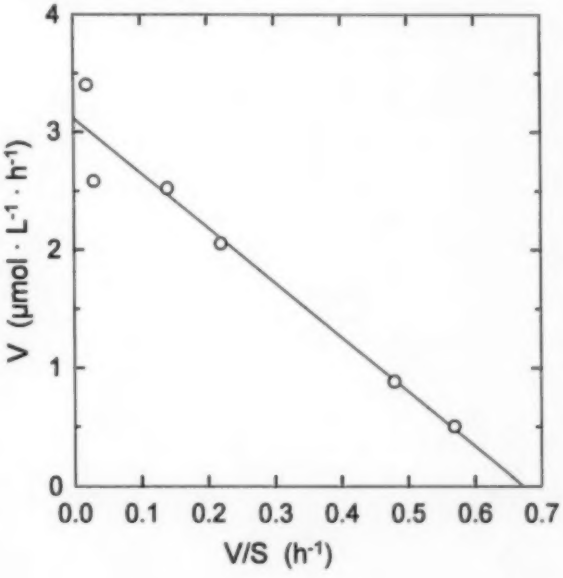
*Uptake was stimulated by nitrapyrin.

those in Lake 227. Even in the nutrient-depleted surface (0–4 m) waters of Lake St. George, DIN is about 10 µM, while below 4 m, levels increase above 50 µM. Amaral and Knowles (1995*a*) showed that thin bands of methanotrophic activity develop within diffusion-controlled agarose columns that are not nitrogen limited and, therefore, may simulate the situation in Lake St. George. Such bands developed where the supply rates of O_2 and CH_4 were in the ratio of 1.5–2.0. Furthermore, Amaral and Knowles (1995*b*) showed that, in such systems, denitrification can occur associated with the CH_4-oxidizing band or lens, suggesting that a similar association might occur in Lake St. George and result in the apparent sink for nitrogen oxides, which exists near the upper boundary of the hypolimnion (Bédard and Knowles 1991).

The predominance of methanotrophs over chemoautotrophic ammonia oxidizers in the process of CH_4 oxidation at the thermocline of Lake St. George is indicated by the following findings. First, the CH_4 oxidizers show a high affinity for CH_4. Second, ratios of CH_4 oxidation to allylthiourea-sensitive CO oxidation are high. Third, CH_4 oxidation is highly sensitive to picolinic acid.

The K_m value for CH_4 oxidation obtained here (4.6 µM) is very close to that obtained in Lake 227 (4.7 µM) by Rudd and Hamilton (1975). It is near the low end of the range of half-saturation constants (1–66 µM) determined for methanotrophs in pure culture and lower than all such values from ammonia oxidizers (Bédard and Knowles 1989) but higher than the 68–460 nM observed for CH_4 oxidation in Saanich Inlet (Ward and Kilpatrick 1990).

Fig. 4. The effect of CH_4 concentration (S) on CH_4 oxidation rate (V) of water from 8.25 m on September 3, 1985. The range of concentrations included is 0.9–180 µM. The data were plotted according to the following linear transformation of the Michaelis–Menten equation: $V = V_{max} - K_m(V/S)$. The equation of the line of best fit as determined by regression analysis is $V = 3.09 - 4.55(V/S)$ ($r^2 = 0.94$, $p < 0.05$).



Ratios of CH_4 oxidation to allylthiourea-sensitive CO oxidation obtained in Lake St. George are typical of ratios measured in pure cultures of methanotrophs and significantly higher than in chemoautotrophic ammonia oxidizers (Jones et al. 1984). The ratios obtained in Lake St. George may in fact be even somewhat low because, in the present study, CO and CH_4 were added to assay bottles in approximately similar concentrations, whereas Jones et al. (1984), in their work on pure cultures, employed CH_4 in concentrations five times higher than CO. The levels of CH_4 and CO used here and in the studies of Jones and Morita (1983, 1984) are below saturation for both methanotrophs and ammonia oxidizers (Bédard and

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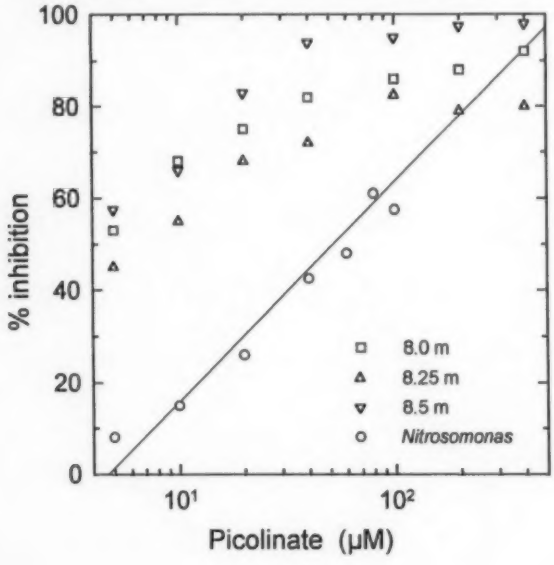
Fig. 5. Inhibition by picolinic acid of NO_2^- production in cell suspensions of *Nitrosomonas europaea* and of CH_4 oxidation in water samples from the depths indicated on September 3, 1985. The line is the best fit for *Nitrosomonas* data by regression analysis.



Knowles 1989) such that activity increases linearly with substrate concentration. Although the ratios observed appear to indicate a methanotroph-dominated system, this interpretation may be complicated by the presence of the alternative substrate NH_4^+ in the lake water. Ammonia could inhibit oxidation of CH_4 and CO, although this may be significant only at concentrations of NH_4^+ considerably higher than those in the metalimnion of Lake St. George (Ward and Kilpatrick 1990). Another potential problem is that, at low levels (<700 μM), NH_4^+ can stimulate CH_4 oxidation and depress CO oxidation by ammonia oxidizers (Jones and Morita 1983, 1984).

Picolinic acid is reported to inhibit methanotrophic activity much more than it inhibits activity of chemoautotrophic nitrifiers (Salvas and Taylor 1984; Megraw and Knowles 1990). Methane oxidation in Lake St. George is highly sensitive to picolinic acid, and inhibition curves obtained from lake-water samples closely resemble the curve determined for the obligate methanotroph *Methylosinus trichosphorium* OB3b where the apparent K_i was 7.6 μM (Megraw and Knowles 1990). In the present study, the apparent K_i for inhibition of NH_4^+ oxidation by *N. europaea* was nearly one order of magnitude higher than in *M. trichosporium* OB3b. Despite the greater sensitivity of methanotrophs to picolinic acid, it may still be difficult to select a concentration of inhibitor that will block methanotroph activity but leave chemoautotrophic ammonia oxidizers unaffected. Allylsulfide, which inhibits nitrifiers much more than it inhibits methanotrophs (Roy and Knowles 1995), may have potential as a differential inhibitor in studies such as these.

The strong inhibition of CH_4 oxidation in the lake water by the chelator allylthiourea might suggest that the methanotrophs possessed the particulate, copper-containing form of CH_4 monooxygenase and that the lake water is thus copper sufficient. However, this remains to be confirmed.

Oxidation of CO in water is believed to be effected not so much by carboxydotrophic bacteria (which have apparent K_m values >0.5 μM) but by cooxidation by ammonia oxidizers (apparent K_m 97–200 nM) and to a lesser extent methanotrophs (Conrad 1988). The profiles of CH_4 and CO oxidation activities, together with other data presented, suggest that methanotrophs were responsible for the CO oxidation activity at the assay concentrations of ca. 80 nM. We were not able to obtain good data for in situ concentrations of CO, so no conclusion can be made about the ecological significance, if any, of this activity.

Nitrapyrin-sensitive bicarbonate uptake is often used as a measure of the activity of ammonia oxidizers (Billen 1976), but incorrect estimates might be obtained if methanotrophs are present, because nitrapyrin also inhibits bicarbonate uptake by these organisms (reviewed in Bédard and Knowles 1989). It seems that an important part of the high rate of nitrapyrin-sensitive bicarbonate uptake observed at the CH_4 oxidation maximum in Lake St. George is catalyzed by methanotrophs, not ammonia oxidizers.

The metalimnetic O_2 maximum observed in both 1984 and 1985 was correlated with a large population of single-celled cyanobacteria possibly of the genus *Chroococcus*, representatives of which were abundant in the lake in other years (McQueen and Lean 1987). The numbers of other bacteria in the metalimnion and upper hypolimnion were greater than those in the epilimnion, and it is possible that methanotrophs could contribute significantly to metalimnetic bacterial biomass in Lake St. George.

We conclude that, based on the CH_4 oxidizers' affinity for CH_4, the ratio of CH_4 oxidation to allylthiourea-sensitive CO oxidation, and their sensitivity to picolinic acid, methanotrophs were major contributors to consumption of CH_4 and O_2 in the metalimnion of Lake St. George.

Acknowledgments

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Visualizing catch–age analysis: a case study

Laura J. Richards, Jon T. Schnute, and Norm Olsen

Abstract: Catch–age analysis has become a standard tool for modern stock assessment. Widely available graphical techniques can be customized to provide an intuitive framework for this complex procedure. We illustrate a graphical approach using data from a stock of Pacific ocean perch (*Sebastes alutus*). These data encompass typical problems in stock assessment, with missing data for some years and changes in age determination and survey methodologies midway through the time series. Preliminary visual inspection of the raw data reveals important features that must be accommodated in the model development. We tailor a state–space model to address these requirements and provide appendices that completely define the model and its statistical application. We suggest graphical methods for evaluating model output, including residuals and standard errors of population biomass. Such portrayals of uncertainty help define realistic limits to the information inherent in fishery data.

Résumé : L'analyse des prises en fonction de l'âge est devenue un outil standard pour l'évaluation des stocks à l'heure actuelle. Des techniques graphiques largement diffusées peuvent être adaptées pour fournir un cadre de travail intuitif pour accomplir cette tâche complexe. Nous illustrons une approche graphique à l'aide de données provenant d'un stock de sébastes à longue mâchoire (*Sebastes alutus*). On trouve dans ces données des problèmes typiques liés à l'évaluation des stocks, à savoir des données manquantes pour certaines années et des changements dans les méthodes de détermination de l'âge et dans les méthodes d'établissement des relevés survenus à mi-chemin pendant la série chronologique. Une inspection visuelle préliminaire des données brutes révèle des caractéristiques importantes dont on doit tenir compte dans l'élaboration du modèle. Nous avons adapté un modèle d'espace d'états pour répondre à ces exigences et fournissons des annexes qui définissent entièrement le modèle et son application statistique. Nous suggérons des méthodes graphiques pour évaluer le rendement du modèle, y compris les résidus et les écart-types de la biomasse de la population. De telles représentations de l'incertitude aident à définir des limites réalistes pour l'information inhérente aux données sur les pêches.
[Traduit par la Rédaction]

Introduction

Catch–age analysis has become a standard procedure for assessing the world's major fishery resources. Many variants have been developed, ranging from simple deterministic back-calculations (Gulland 1965; Pope 1972) to statistical models that encompass uncertainty in the underlying data and model parameters (Fournier and Archibald 1982; Deriso et al. 1985; Gudmundsson 1994; Schnute and Richards 1995; McAllister and Ianelli 1997). The modern methods combine information from a variety of sources to make inferences about stock population dynamics.

In this paper, we demonstrate how graphical techniques can contribute to catch–age analysis. Our case study illustrates the potential of visualization tools for examining input data and for interpreting analytical results. Graphical methods provide clues to the information content of the input data. They also help to identify specific features of the data that must be accommodated in the underlying model structure. While graphical tools have always been important in fisheries, recent technical developments in computer hardware and software allow a high level of graphical sophistication. Cleveland (1993) describes a collection of graphical tools that have become embedded in modern statistical software packages. We apply a selection of these tools to catch–age analysis.

To provide a practical example, we examine a particular data set in the context of state–space models developed by Schnute and Richards (1995). Our data encompass typical problems in stock assessment, with missing numbers for some years and changes in age determination and survey methodologies midway through the time series. Although our analysis highlights specific aspects of the example, figures in this paper could aid in the choice of suitable graphical displays for many catch–age analyses. We select graphical tools with sufficient flexibility to portray the variety of features commonly found in realistic data sets.

Section 1 provides background and preliminary analysis of the input data. Our example pertains to the Pacific ocean perch (*Sebastes alutus*) fishery in Goose Island Gully, a submarine canyon located in southern Queen Charlotte Sound, British Columbia, Canada. Section 2 shows how the general model of Schnute and Richards (1995) can be tailored to accommodate specific features of the Pacific ocean perch data. We use graphical methods to assess model output, including residuals and standard errors of population biomass. The visual link between input data and model output guides analytical development and fosters intuitive understanding of the results.

Our intent is to demonstrate how graphical methods can convey a complex catch–age analysis. We confine technical details to appendices, where Appendix A defines a complete catch–age model and Appendix B presents the statistical theory required for estimating parameters and their associated uncertainty. Discussion in the body of the paper provides an intuitive framework for the technical analysis.

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Fig. 1. Pacific ocean perch reported trawl catch (tonnes) from Goose Island Gully by nation (top panel) and the corresponding age proportions sampled from the catch for recruited ages of 7 years and older (bottom panel). The radius of a circle is proportional to the measured age proportion, where data are scaled to sum to 1 down each column and ages 29 years and older are grouped. The vertical line distinguishes age data obtained by surface readings of otoliths (1963–1976) from later break and burn readings (1977–1995). No age proportion data are available for 1986 or 1988.

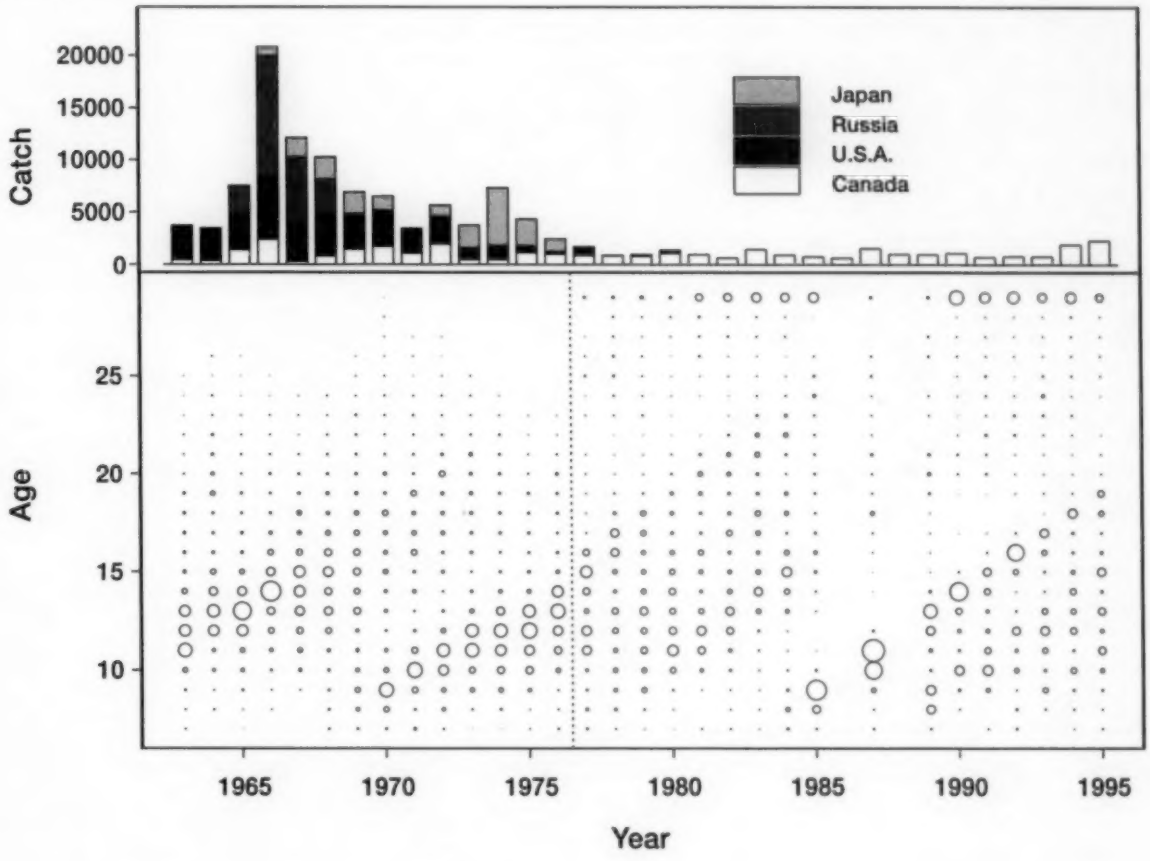

Table 1. Data sets used for this analysis of the Goose Island Gully Pacific ocean perch stock.

Years	Data set
1963–1995	All-nation annual trawl catch
1963–1976	Age proportions in the catch; surface ageing method
1977–1995	Age proportions in the catch; break and burn method
1965–1984	Ten research vessel survey biomass estimates
1984–1995	Four charter vessel survey biomass estimates
1976–1995	Tonnages for individual fishing vessels
1976–1995	Landed catch and effort by fishing trip and vessel
1963–1995	Weight-at-age from length-at-age and length–weight conversion
1977–1995	Maturity-at-age

1. Biological and fishery data

For stocks with a long history of exploitation and study, the information available for catch–age analysis may be voluminous and complex. Any analysis begins with a thoughtful compilation of the data. Table 1 provides a description of the data that we use for the Pacific ocean perch example here. These data include sources used in most catch–age analyses, plus additional information on vessel tonnages and individual fishing trips.

The first three items listed in Table 1 constitute the core data for any catch–age analysis: fishery catch and proportions at age. Figure 1 provides a compact representation of these key data. A stacked bar plot (upper panel) portrays the annual catch by nation. Age proportions in the catch are plotted as circles (lower panel), where the radius of each circle is proportional to the corresponding age proportion. Within a given year, age proportions sum to 1 for recruited ages (age 7 years and older). Apparently, the fishery is supported by occasional strong year-classes that can be identified as progressions of large circles ascending from left to right.

The catch data in Fig. 1 show that the stock has been subjected to two very different periods of exploitation: a large multinational fishery until the mid-1970s, followed by a comparatively small Canadian fishery. The fishery actually began in the 1950s by U.S. vessels, but we do not have matching age data prior to 1963. Large catches were harvested by Soviet and

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Japanese fleets during the late 1960s and early 1970s, prior to extended jurisdiction. Other rockfish (*Sebastes* spp.) stocks along the Pacific coast of North America were also targeted by Soviet and Japanese fleets at this time. Several studies in the early 1980s (Ketchen 1981; Kimura 1981; Archibald et al. 1983) indicated depletion of the Goose Island Gully Pacific ocean perch stock by the late 1970s. The subsequent Canadian fishery was therefore restricted by low annual quotas and other management measures.

Figure 1 illustrates several essential features of the input data. Apparently, large catches during the period 1963–1976 included few fish older than 25 years. Ages for these years were obtained from surface readings of otoliths (Archibald et al. 1983). Subsequent readings (1977–1995) by the break and burn method (Chilton and Beamish 1982) revealed the presence of older fish (up to 79 years) and suggested that surface readings underestimate the true age for Pacific ocean perch older than about 15–16 years (Stanley 1987). Unfortunately, the early otolith samples were destroyed in a fire before they could be reexamined by the break and burn method.

Additional ageing error among younger ages (10–15 years) may be indicated in Fig. 1. For example, relatively large proportions of ages 11–13 years in 1974–1976, respectively, appear adjacent to similarly large proportions for ages ±1 year within each year. Such patterns could result from ageing error and thus wrongly suggest three consecutive strong year-classes.

Other features of the age structure data in Fig. 1 illustrate how model design must be tailored to the input data. For example, the modal age-class within a year is always older than the age-class at first recruitment, indicating that availability to the fishery increases with age for younger ages. Consequently, an appropriate model for these data, as in most catch–age analyses, must allow selectivity by the fishery to vary with age. Similarly, because no age data are available for 1986 and 1988, the model's observation equations must allow for missing data.

Catch and age proportions alone are generally inadequate to reconstruct the history of a fish population. Most catch–age analyses rely on at least one additional abundance index, a measure related to the size of the underlying fish population. For the example here (Table 1), several research vessel trawl surveys were conducted between 1965 and 1977. Then, in 1984 when the research vessel was slated to be decommissioned, a calibration survey was conducted in conjunction with a chartered commercial vessel. Subsequent surveys in 1994 and 1995 were conducted by chartered commercial vessels (Hand et al. 1995; Yamanaka et al. 1996). The resulting data, portrayed in Fig. 2 (upper panel), include problems typical of indices available for long-term fisheries. The time series contains large gaps and a methodology change that necessitates calibration between two indices.

Confidence intervals in Fig. 2 are determined from standard application of sampling theory for a stratified random design (e.g., Cochran 1977, chap. 5). The confidence interval is missing for the first survey because only one sample tow was completed in one of the four survey depth strata for that year. At best, these intervals provide a minimal estimate of the true survey variance; they incorporate only the variation in catch rates among survey tows within a given year.

Figure 2 requires a conversion factor to scale charter vessel data on the right-hand axis relative to research vessel data on the left-hand axis. By methods discussed below, a model is used to estimate this factor. Input data in Fig. 2 (upper panel) comprise research and charter vessel index estimates (circles) and corresponding 95% confidence intervals (vertical lines). Other components of the figure, derived from model results, are discussed in the next section. If the scale factor between left and right axes is accepted, Fig. 2 indicates that the population has dropped from historic highs in the 1960s to low values in the 1980s, with a possible increase in the 1990s.

For our example, we are fortunate to have indices based on designed surveys. In other circumstances, catch and effort data from the commercial fishery are used to develop an abundance index based on catch per unit effort (CPUE). Hilborn and Walters (1992, pp. 175–192) provide several reasons for discarding CPUE as an abundance index, including spatial and temporal responses by fishers to the abundance of fish. For the Pacific ocean perch fishery, these factors are compounded with temporal changes in the operation of the fishery. Consequently, we reject CPUE as a potential abundance index in our analysis.

Figure 3 summarizes some aspects of the changing Pacific ocean perch fishery during the last two decades. Box plots show the Pacific ocean perch catch on individual fishing trips between 1976 and 1995, where the solid line indicates the total number of fishing trips for each year. The number of fishing trips increased after 1985, with a corresponding decrease in median trip catch. Given annual catches that are similar throughout this period (Fig. 1), the box plots illustrate how fishing operations have shifted from a few large trips to many small trips each year. This shift can be directly attributed to regulatory changes. More vessels participated after the mid-1980s, and managers introduced trip limits (quotas on individual trips) to distribute fishing effort more evenly over the 12-month fishing season. Trip limit controls on the Goose Island Gully Pacific ocean perch fishery became increasingly stringent during the early 1990s. Thus, trip limit controls can be expected to alter any potential relationship between CPUE and fish abundance. Indeed, Richards (1994) found a positive association between a CPUE index and the magnitude of the trip limit for this and another stock.

2. Model analysis and results

The data described in section 1 presumably reflect features of the underlying population dynamics of Pacific ocean perch. For example, Fig. 1 shows the progression of strong age-classes as they grow older. To combine various data sources into a coherent framework, we require a model that relates available observations to the hidden biological system. Appendix A provides complete model details, where we tailor the state–space approach of Schnute (1994) and Schnute and Richards (1995) to accommodate the Pacific ocean perch data.

Briefly, the model describes a population that experiences annual recruitment and natural mortality, in addition to removal of catch by the fishery. Various known data can be considered controls on the population dynamics. For example, the catch biomass constitutes a known human control. Similarly, average fish weights and maturities that depend on age can be considered natural biological controls. The model uses weight information to convert between fish numbers and biomass. Maturity schedules are needed to calculate the mature spawning biomass.

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Fig. 2. Top panel: Point estimates and 95% confidence intervals for the Pacific ocean perch biomass index (1000 tonnes) obtained by swept-area trawl surveys of Goose Island Gully using a research vessel (solid circles) and chartered commercial vessels (open circles). The two lines give the estimated index trend from model formulations with $M = 0.07$ (estimated) or $M = 0.05$ (fixed). Model estimates for the research and charter indices differ by a scale factor that depends on the model formulation. Circles and error bars correspond to the choice $M = 0.07$. Rescaled charter estimates with $M = 0.05$ are indicated by the open triangles. Bottom panel: Model residuals (log observed minus log expected in (L.6) and (L.7)) for the fit of the survey index data with $M = 0.07$ (estimated).

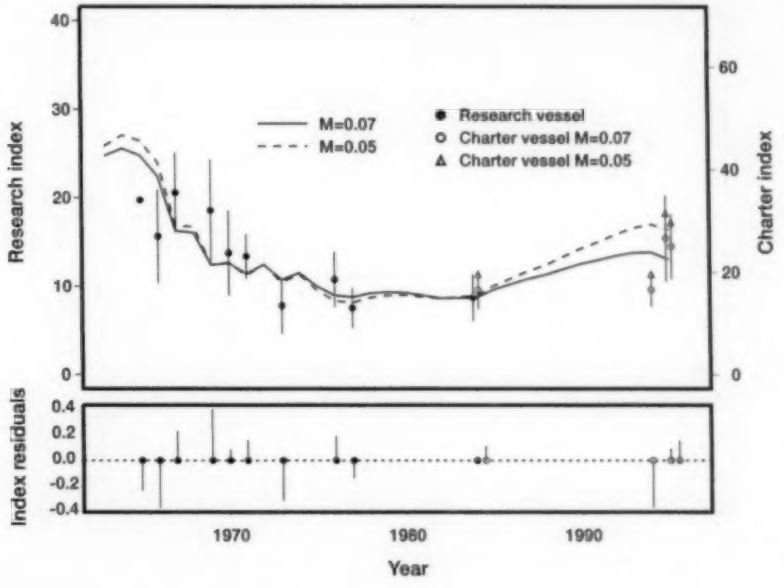

Fig. 3. Box plots of Pacific ocean perch landed catch on fishing trips to Goose Island Gully by Canadian vessels between 1976 and 1995. Trips are included for which the Pacific ocean perch catch is 20% or more of the total catch. The solid line gives the corresponding number of fishing trips. Boxes illustrate the interquartile range of trip catches with the median represented by the solid bar. Whiskers are drawn to the nearest catch value not beyond 1.5 times the interquartile range. Extreme catch values are indicated by solid points.

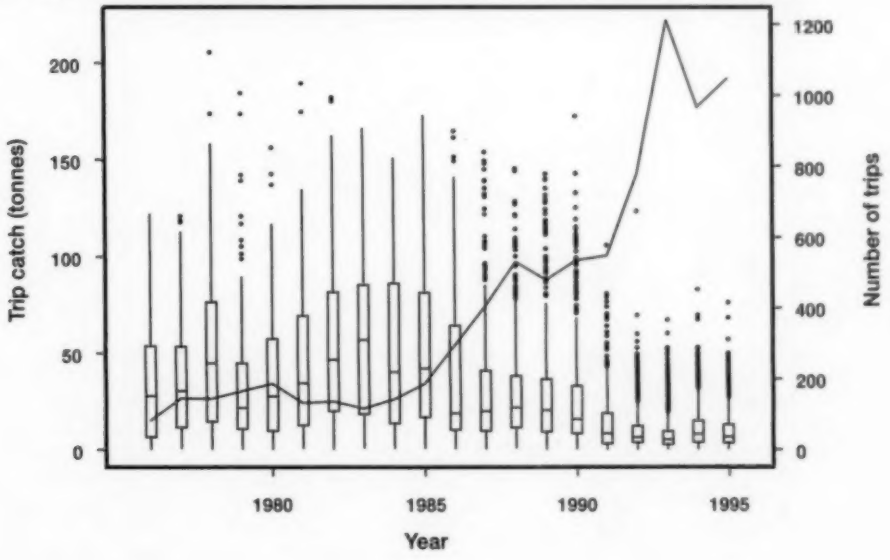

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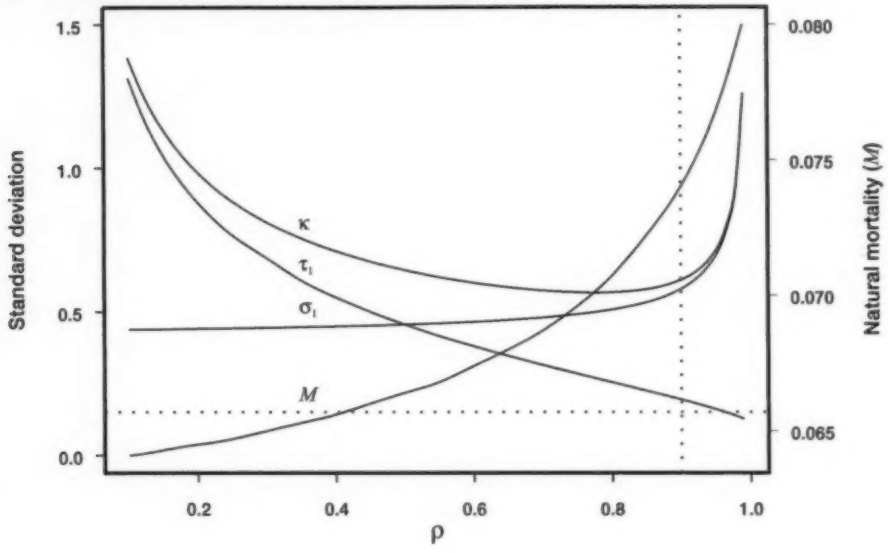
Fig. 4. Estimates of model standard deviations κ, σ_1, and τ_1 and the natural mortality M for a range of fixed values of the variance ratio ρ. The horizontal grid line indicates the value τ_1 that corresponds approximately to the measured standard deviation of the survey data. The vertical grid line indicates the fixed value $\rho = 0.9$ selected for subsequent model analysis.



Controls influence the system dynamics; by contrast, observations merely reflect the system's current state. From this point of view, we have only two sets of observations: the age proportions in Fig. 1 and the index data in Fig. 2. The latter can be subdivided into distinct indices from research and charter vessel surveys. The model includes equations that relate these data, observed with measurement error, to unknown numbers of fish in the underlying population. The model's main purpose is to reconstruct the population history from all known controls and observations. This reconstruction must also take account of statistical estimation error due to uncertain measurements and other sources of variability.

Intuitively, the model extends a classical removal experiment, where removals of fish are related to declines in an abundance index. In the present context, fish are added to the initial age-class through recruitment and removed from all age-classes by the fishery. The model attempts to explain observed age proportions and index data by adjusting recruitment and other unknown biological parameters to account for known fishery removals.

Our model does not include fishing mortality parameters, as in the Baranov equations (Ricker 1975, p. 13). Instead, we subtract the catch directly in our dynamic equations. We prefer this formulation because it requires fewer parameters and ensures that the population is reduced by the actual biomass of fish removed. Other model formulations (Fournier and Archibald 1982; Deriso et al. 1985; Methot 1989, 1990; McAllister and Ianelli 1997) assume that fishery removals are measured with error, so that the true catch data (treated as unknown controls) vary randomly above or below the observed numbers. This allows true catches within the model to differ from observed catches.

The likelihood for the catch–age model in Appendix A conforms to the errors-in-variables paradigm discussed by Schnute (1994). Parameters include recruitments R_t in year t, and we assume that these follow a lognormal autoregressive process with standard error σ_1. This process error determines the predominant source of natural variability in population abundance. Similarly, we assume lognormal measurement error in the index data with standard error τ_1. Schnute and Richards (1995) show that these two sources of error complement each other in the model. Intuitively, apparent variations in abundance can be explained primarily through variability in recruitment (high process error σ_1) or indexing (high measurement error τ_1). Such ambiguities are inherent to most models that include both measurement and process error (Kendall and Stuart 1979, chap. 29).

We resolve this ambiguity in a standard way by fixing the variance ratio

$$(1) \qquad \rho = \frac{\sigma_1^2}{\sigma_1^2 + \tau_1^2}$$

between recruitment and total variance

$$(2) \qquad \kappa^2 = \sigma_1^2 + \tau_1^2 .$$

A value of ρ near 1 corresponds to high variance in recruitment and low variance in the model fit to the survey index. Conversely, a low value of ρ corresponds to near constant recruitment and high variance in the survey index fit.

We begin our analysis by identifying an appropriate choice of fixed ρ for subsequent model runs. This choice is analogous to the choice of likelihood weights or emphasis factors in other catch–age models, such as stock synthesis (Methot 1989, 1990). An advantage of our implementation is that we can attribute a biological interpretation to this choice.

Given the data here, Fig. 4 shows how various parameter estimates depend on the choice of ρ. The composite model error κ decreases to a minimum near $\rho = 0.8$ and then increases

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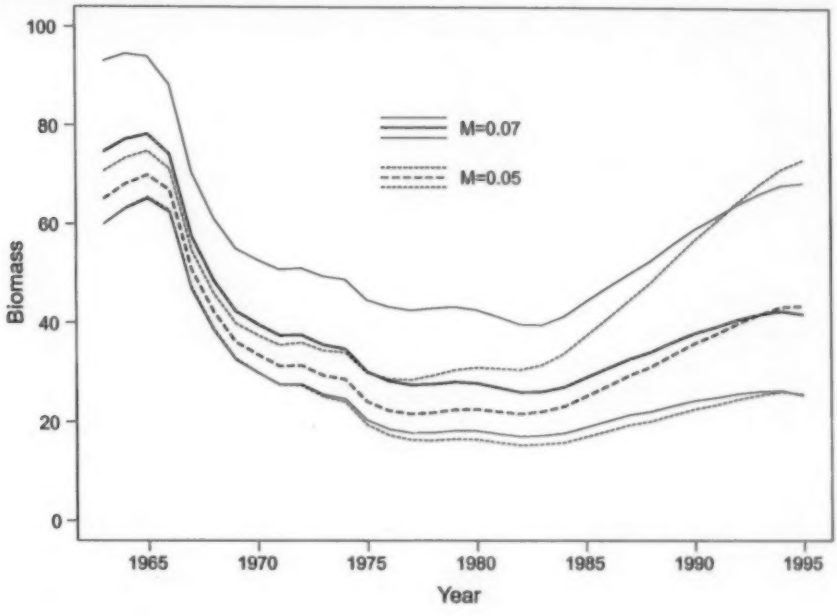
Fig. 5. Model estimates and 95% confidence intervals with $M = 0.07$ (estimated) and $M = 0.05$ (fixed) for Pacific ocean perch exploitable biomass (1000 tonnes) in Goose Island Gully between 1963 and 1995.



sharply. Estimates of σ_1 are relatively insensitive to choices of ρ smaller than about 0.8, while estimates of τ_1 decrease with increasing choices of ρ. The survey index data have a coefficient of variation of about 15% (Yamanaka et al. 1996), corresponding approximately to the estimate $\tau_1 = 0.15$, indicated by the horizontal grid line in Fig. 4. We selected $\rho = 0.9$ (vertical grid line) for subsequent runs to achieve a low estimate of κ and an estimate of τ_1 similar to the minimal standard error in the survey data.

Classical catch–age analyses usually assume that the natural mortality rate M is known a priori. In our model, M is just one of the parameters to be estimated from available data. Figure 4 shows how the maximum likelihood estimate of M (from 0.064 to 0.080) varies with the choice of ρ in the range (0.10, 0.99). In particular, the estimate of M is 0.073 when $\rho = 0.9$ and approaches an asymptote near 0.064 as ρ becomes small. Previous assessments of Pacific ocean perch have assumed a fixed value $M = 0.05$ (Archibald et al. 1983; Ianelli and Heifetz 1995; Richards and Olsen 1996), somewhat smaller than the model point estimates for any choice of ρ. Thus, with our choice $\rho = 0.9$, we consider two estimation scenarios in which M is estimated ($M = 0.07$ to the first significant digit) or fixed at the value used historically ($M = 0.05$).

For most fishery data sets, estimates of M and population size tend to be positively correlated. In the Pacific ocean perch application, we obtain higher biomass estimates for most of the time series with $M = 0.07$ (estimated) than with $M = 0.05$ (fixed), although the final estimates are similar (Fig. 5). As discussed in conjunction with Fig. 2, model results indicate that Pacific ocean perch biomass declined during the late 1960s and early 1970s from historical highs in the 1960s. Biomass was relatively stable during the late 1970s and early 1980s and then increased through the early 1990s. Interpretation of the biomass trend depends on M; with M fixed, the recent biomass increase is more pronounced than with M estimated. Furthermore, although 95% confidence intervals become broader for more recent estimates, biomass estimates through the 1970s appear more certain (exhibit narrower confidence intervals) with M fixed than with M estimated. The restrictive assumption $M = 0.05$ eliminates one parameter from the estimation process, resulting in biomass estimates with exaggerated certainty. Interestingly, the lower confidence interval is similar for both trajectories; the history of catch removals effectively constrains the lower bound of plausible population biomass estimates.

Figure 2 (upper panel) relates the biomass trajectories in Fig. 5 to the two observed survey indices. To display this comparison, we convert biomass amounts to index units using two scale factors (q_1 and q_2 in Appendix A) estimated by the model, where one factor applies to each of the two survey methods. These scale factors allow biomass to be expressed in either research or charter vessel survey units. Furthermore, they enable conversion from one survey unit to the other. In this way, the two axes on the left and right sides of Fig. 2 can be scaled to each other and the biomass trajectory can be plotted with reference to both surveys simultaneously. Because surveys occur during the fishery, a small correction in the model takes account of the harvested biomass prior to the survey measurement.

After the adjustments above, Fig. 2 shows the biomass trajectory with $M = 0.07$ (estimated) in relation to research vessel surveys (solid circles, scaled on the left) and charter surveys (open circles, scaled on the right). Another line shows the biomass trajectory for $M = 0.05$ (fixed) in relation to research vessel survey units (left axis). For this line, the right axis scale does not apply because the relative adjustment between survey

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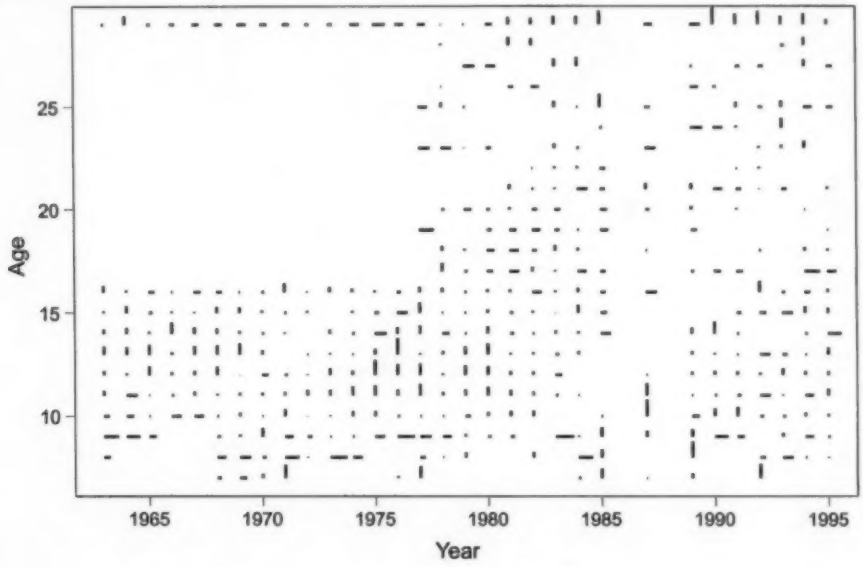
Fig. 6. Age proportion residuals (L.8) for the model with $M = 0.07$ (estimated). Residuals are proportional to the length of the line segment, where positive residuals (observed larger than predicted) are oriented vertically upward and negative residuals are oriented horizontally to the right. Blank spaces result from combined age groups, where observed age proportions smaller than 2% of the sample are grouped consecutively within a year. Surface-read age proportions are grouped for ages 17 years and older, while break and burn age proportions are grouped for ages 29 years and older.



indices depends on the chosen model. Furthermore, taking account of the different adjustment between survey methods for the case $M = 0.05$, the charter surveys are plotted in research vessel survey units as open triangles, somewhat higher than the open circles. Thus, the higher biomass trajectory after 1980 in the case $M = 0.05$ results from a different scaling between research and charter vessel surveys.

Confidence intervals associated with observed survey indices (solid and open circles) in Fig. 2 have been obtained directly from the survey data. The model trajectory when $M = 0.07$ intersects these intervals for most years of survey data. This indicates at least some consistency between empirical survey error and model fit, although we recognize that the empirical survey error underestimates the actual error. To examine the fit further, we consider the index residuals (Fig. 2, lower panel; equations (L.6) and (L.7) in Appendix A) for the case $M = 0.07$ (estimated). These vary with no obvious consistent trend.

Figure 6 represents the age proportion residuals (equation (L.8) in Appendix A) for the case $M = 0.07$ (estimated). Vertical or horizontal line segments indicate positive or negative residuals, respectively, where each directed line segment points either upward or to the right. We use this graphical device to conserve vertical space in the figure and take advantage of horizontal space between columns. Generally, larger residuals correspond to younger age-classes. The model tends to overestimate the proportion of age 8–9 fish (negative residuals) and underestimate the proportion of age 11–13 fish, particularly for the 1963–1980 data. Proportions in the accumulator age-class tend to be overestimated for surface ages and underestimated for break and burn ages. This analysis suggests that a different selectivity function (equation (M.7) in Appendix A), possibly with more than two parameters, may provide a better fit to the data.

Distinct time trends are apparent in the estimated recruitment series (Fig. 7), with above-average year-classes from the 1950s, early 1960s, and late 1970s through the early 1980s. Peak recruitments are associated with the 1952, 1961–1962, 1976, and 1980 year-classes. Recruitment trends are to some extent related to ageing error; the model cannot distinguish a strong year-class with ages known to say ±1 year from two or three consecutive above-average year-classes. However, the model does provide evidence that recent recruitments have been somewhat higher than recruitments experienced during the early 1970s. It is these recent recruitments, combined with low catches, that account for the increase in biomass following an apparent low in the early 1980s.

Forecasts of potential future harvests depend on forecasts of recruitment, which must be dependent on spawner biomass, at least at lower spawner biomass levels. We could find no evidence for reduced recruitment at low spawner biomass within the observed spawner biomass range (Fig. 8). However, the joint 95% confidence intervals are extremely broad for the age-7 recruitment in year t and the corresponding spawner biomass in year $t - 7$. Thus, estimation error is sufficiently large to mask any spawner–recruit relationship.

Discussion

A serious catch–age analysis cannot be described completely without some degree of complexity. For example, the model defined in Appendix A requires notation for various data, parameters, and system states (Table A.1). Numerous equations describe the initial population, system dynamics, and

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Fig. 7. Estimates (solid circles) and 95% confidence intervals for the biomass of age-7 recruits (1000 tonnes) from year-classes 1946–1988. Lines denote related estimates of spawner and exploitable biomass (1000 tonnes), where all estimates are obtained from the model with $M = 0.07$ (estimated).

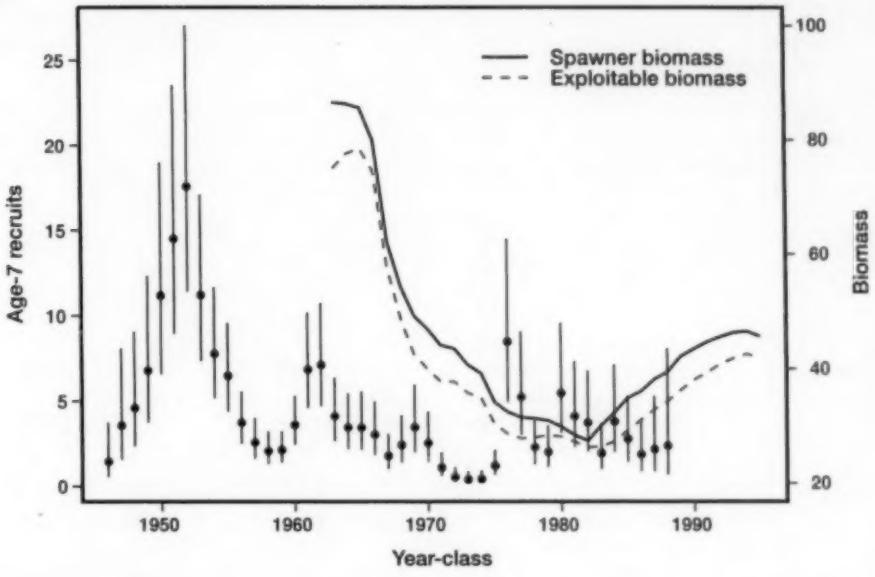
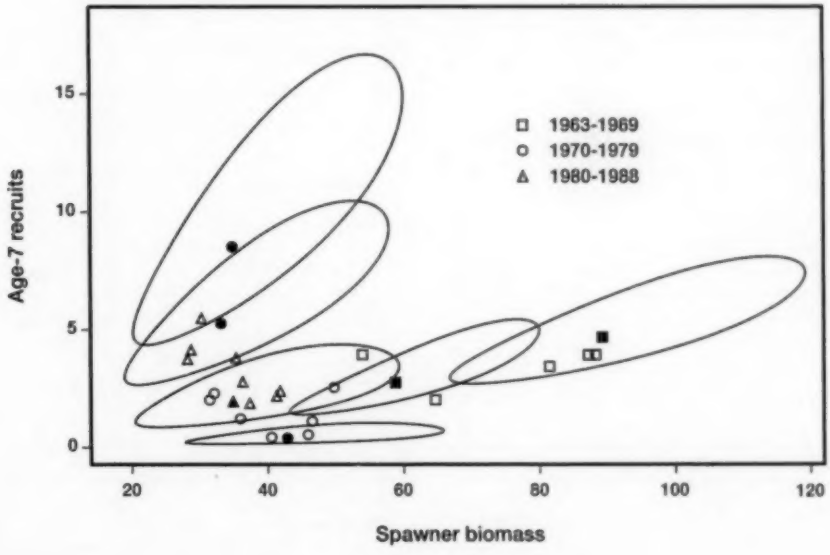


Fig. 8. Relationship between estimates of age-7 recruits (1000 tonnes) and spawner biomass (1000 tonnes) 7 years earlier, with 95% joint confidence intervals for selected points (solid symbols) for the model with $M = 0.07$ (estimated). Different point types distinguish decades of year-classes.



estimated observations (Table A.2). A complex likelihood function (Table A.3) compares observed data with estimates. Finally, statistical theory (Appendix B) must be used to obtain estimates and measures of uncertainty for various quantities of interest. This edifice must seem fairly daunting to a general audience unfamiliar with statistical and mathematical terminology.

In this paper, we have used graphical devices to facilitate an intuitive understanding of such a complex analysis. We have confined technical material to appendices and included almost no mathematics in our discussion of the concepts that underlie catch–age analysis. Although we have dealt primarily with the Pacific ocean perch example, our intent has been to illustrate procedures widely applicable to fishery data analysis.

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We believe that carefully crafted graphics can be informative to both analysts and general readers. Furthermore, analysts steeped in the technical details might easily overlook important features of the input data and estimated output. For example, many catch–age analyses do not include measures of uncertainty comparable with those displayed in Figs. 5, 7, and 8.

Our figures incorporate widely available graphical techniques, including bar plots, box plots, symbol plots, line plots, and scatter plots. However, none of the figures can be generated automatically with standard graphical software tools. In all cases, the graphics must be tailored to address specific presentation requirements. These result both from features of the input data (e.g., missing data, data scaling) and from display considerations (e.g., plot types, line types, plotting symbols, multipanel plots). In general, analysts need to modify generic tools to suit a specific application.

We selected our residual plot (Fig. 6) from a large number of potential alternatives. Our final choice was dictated by *Journal* constraints for black and white reductions. In a larger size format, or in a problem with fewer dimensions, a dot could be used to indicate the 0 value as in Fig. 2. When we examined this approach for our residual data, we found that the dots hid much of the information in the line segments. We also attempted to reproduce Fig. 6 with solid and open circles, a variant of the symbol plot in Fig. 1. However, with this technique, the solid circles tended to dominate the plot. A symbol plot may be more appropriate in a color format, where positive and negative residuals can be represented by circles of different colors.

We emphasize that the figures here only hint at possibilities for other analyses. For example, if many countries participate in a fishery, a stacked bar plot such as Fig. 1 may be unintelligible. A reviewer suggested that a cumulative proportion of the total catch for each country by year might produce a graphical display that is easier to interpret for this situation. To some extent, visual representations constitute an art form that reflects the analyst's taste and creativity.

We generated our figures using the software package S-PLUS (Statistical Sciences Inc. 1995), an implementation of the S language (Becker et al. 1988; Chambers and Hastie 1992; Venables and Ripley 1994). Modern graphical packages such as S-PLUS enable the construction of complex functions from basic graphical components. Rich graphical programming languages allow the fine control of graphical features that we have used here.

Graphics alone, however, are not sufficient for a complete interpretation of fishery data. Catch–age analysis, for example, aims to reconstruct a population history from a record of catch removals, observed age proportions, and abundance indices. A model, such as ours in Appendix A, must be used to link such information within a framework that relates observed data to the underlying fish population. Scientifically, the model consists of assumptions to be examined, tested, and possibly altered. Graphical analysis can aid this process. For example, the residual plot in Fig. 6 suggests that our selectivity assumption (M.7) may be too restrictive. Cleveland (1993) devotes much of his book to analysis of residuals and other aspects of model output.

S-PLUS is not designed to accomplish all the model analysis required here. In the Pacific ocean perch example, with 33 years of data and 11 or more age-classes, the model requires estimates of over 50 parameters. A wide variety of nonlinear estimation software packages are currently available for implementing such analyses (Moré and Wright 1993). The Optimization Technology Center (1996) provides an excellent starting point for an internet search. Appropriate software should readily accommodate models like that in Appendix A, as well as the uncertainty calculations documented in Appendix B. We used the software package AD Model Builder (Otter Research Ltd. 1994) to meet these requirements.

Following the analysis of Schnute and Richards (1995), we have used a comparatively simple model for estimation. For example, we have not included survival process error in the population dynamics. As a result, the model parameter vector (which includes recruitments) completely determines the underlying population history. If we allowed additional sources of error, the estimation process would be more complex and lead to even greater uncertainties than those portrayed in Figs. 5, 7, and 8. Even with our relatively rigid model, large uncertainties exist in the current biomass of Pacific ocean perch. By taking serious account of statistical error inherent in our data, we are forced to realize that our knowledge of the population is rather limited.

The stock–recruitment plot in Fig. 8 demonstrates rather dramatically the consequences of uncertainty in a matter of key biological importance. A stock–recruitment relationship, designed without taking this large uncertainty into account, could lead to dangerously optimistic harvest control policies. More generally, graphical analyses that portray uncertainty can play an important role in defining realistic limits to the information inherent in fishery data.

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Appendix A. Model design

Table A.1 presents notation for a model, similar to that proposed by Schnute and Richards (1995), which is tailored to the Pacific ocean perch example here. Following state–space design principles described by Schnute (1994), our model includes a population state vector $\{N_{at}\}_{a=1}^{A}$ for each year t. Equations (M.13)–(M.17) in Table A.2 define the biological system dynamics for these states, based on the parameter vector Φ in (M.6) and control data (M.3) and (M.4). The controls can be subdivided into human interventions (M.3) and biological characteristics (M.4).

Given the evidence from Fig. 1, the model includes selectivities β_a in (M.7) that vary with age-class a. Moments (M.8)–(M.11) of the state vector, such as the exploitable population P_t and exploitable age proportions u_{at}, depend on these selectivities. The known catch biomass D_t is converted to catch numbers C_t in (M.11), using the average weights w_{at}. Similarly, age-dependent maturities m_a enter the calculation (M.12) of spawner biomass S_t.

Equations (M.18)–(M.21) in Table A.2 relate observed data to the underlying biological system. Following Schnute and Richards (1995), we indicate an estimated observation with a line over the corresponding symbol. Two biomass indices I_{1t} and I_{2t} correspond to the research and charter vessel surveys conducted in various years $\mathbf{T}_1$ and $\mathbf{T}_2$, respectively. Estimated survey numbers are assumed proportional to the underlying exploitable biomass after a known fraction f_{it} ($i = 1, 2$) of the catch D_t has been removed. Similarly, the proportions p_{at} are estimated directly from the exploitable proportions u_{at} calculated within the model.

By definition, accumulator age-classes contain all fish with ages equal to or older than the designated age. In the model here, age-class $a = 1$ corresponds to fish that recruit at age 7 years. To avoid potential bias from otolith surface readings (Fig. 1), we use accumulator age-classes $A' = 11$ and $A = 23$ (actual ages 17 and 29) for fish aged by surface or break and burn readings, respectively. Consequently, in year t, the observed proportions p_{at} in (M.5) have the accumulator class A'_t, given by (M.1). Internal to the model, we use accumulator classes A_t in (M.2), which begin at age A' in year $t = 1$ and advance to a maximum age A in year $t = 1 + A - A'$. The observation equation (M.21) corrects for this difference between observed data and model dynamics.

Table A.2 defines an algorithm for estimating the observations (M.5). The calculation begins with the control data (M.3) and (M.4) and the parameter vector Φ in (M.6), which includes recruitments $\{R_t\}_{t=2-A'}^{T}$. These determine the initial states N_{a1} from (M.13) and (M.14) and the initial moments (M.8)–(M.12). The dynamic equations (M.15)–(M.17) at time $t = 2$ determine N_{a2} from N_{a1}, C_1, and u_{a1}. Following this procedure iteratively gives N_{at} for each time t. Finally, the estimates (M.18)–(M.21)

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Table A.1. Notation for the Pacific ocean perch model.

Symbol	Description
	Indices and index ranges
a	age-class, where $a = 1$ corresponds to age 7
t	year, where $t = 1$ corresponds to 1963
A'	accumulator age-class for surface age data (age 17)
A	accumulator age-class for break and burn age data (age 29)
A'_t, A_t	accumulator age-class for the data and model, respectively, in year t
T'	final year 1976 ($T' = 14$) for surface age data
T	final year 1996 ($T = 33$) of data
$\mathbf{T}_1, \mathbf{T}_2$	sets of years with research and charter vessel surveys
	Data
D_t	observed catch biomass in year t
p_{at}	observed proportion of age-class a fish in the catch from year t
w_{at}	average weight of age-class a fish in year t
m_a	proportion of age-class a fish that are mature
I_{1t}, I_{2t}	biomass estimates from research and charter vessel surveys in year t
f_{1t}, f_{2t}	fraction of catch taken prior to research and charter vessel surveys
	Parameters
Θ, Φ	parameters vectors
α, β_1	selectivity parameters
β_a	selectivity for age-class a
M	natural mortality rate
q_1, q_2	catchability for research and charter vessel surveys
R, γ	autoregressive recruitment parameters
σ_1	standard deviation of recruitment process error
τ_1	standard deviation of survey index measurement
τ_2	standard deviation of age proportion measurement error
κ^2	combined variance $\sigma_1^2 + \tau_1^2$
ρ	variance ratio σ_1^2/κ^2, fixed in the model analysis
	States and state moments
R_t	age-class 1 recruitment in year t
N_{at}	number of age-class a fish at the start of year t
P_t	exploitable population numbers at the start of year t
B_t	exploitable population biomass at the start of year t
S_t	spawner biomass at the start of year t
C_t	number caught in year t
u_{at}	exploitable proportion of age-class a fish in year t

Table A.2. Deterministic model, where the parameter vector Φ and controls (M.3) and (M.4) iteratively define states N_{at}, moments (M.8)–(M.12), and estimates for the observations (M.5).

Accumulator age-classes ($1 \le t \le T$)

(M.1) $$A'_t = \begin{cases} A'; & (1 \le t \le T') \\ A; & (T' < t \le T) \end{cases}$$

(M.2) $$A_t = \min(A' + t - 1, A)$$

Data: controls and observations

(M.3) $D_t\ (1 \le t \le T)$; $f_{1t}\ (t \in \mathbf{T}_1)$; $f_{2t}\ (t \in \mathbf{T}_2)$

(M.4) $w_{at}\ (1 \le a \le A_t;\ 1 \le t \le T)$; $m_a\ (1 \le a \le A)$

(M.5) $p_{at}\ (1 \le a \le A'_t;\ 1 \le t \le T)$; $I_{1t}\ (t \in \mathbf{T}_1)$; $I_{2t}\ (t \in \mathbf{T}_2)$

Parameters

(M.6) $$\Phi = (\alpha, \beta_1, M, q_1, q_2, \{R_t\}_{t=2-A'}^{T})$$

Selectivity

(M.7) $$\beta_a = 1 - (1 - \beta_1)\left(\frac{A - a}{A - 1}\right)^{\alpha}$$

State moments ($1 \le t \le T$)

(M.8) $$P_t = \sum_{a=1}^{A_t} \beta_a N_{at}$$

(M.9) $$B_t = \sum_{a=1}^{A_t} \beta_a w_{at} N_{at}$$

(M.10) $$u_{at} = \beta_a N_{at} / P_t;\ (1 \le a \le A_t)$$

(M.11) $$C_t = D_t / \sum_{a=1}^{A_t} u_{at} w_{at}$$

(M.12) $$S_t = \sum_{a=1}^{A_t} m_a w_{at} N_{at}$$

Initial states ($t = 1$)

(M.13) $$N_{a1} = R_{2-a} e^{-M(a-1)};\ (1 \le a < A')$$

(M.14) $$N_{A1} = R_{2-A'} \frac{e^{-M(A'-1)}}{1 - e^{-M}}$$

State dynamics ($2 \le t \le T$)

(M.15) $$N_{1t} = R_t$$

(M.16) $$N_{at} e^{-M}\,[N_{a-1,\,t-1} - u_{a-1,\,t-1} C_{t-1}];\quad (2 \le a \le A_t;\ 2 \le t \le 1 + A - A')$$

(M.17) $$N_{At} = e^{-M}[N_{A-1,\,t-1} + N_{A,\,t-1} - (u_{A-1,\,t-1} + u_{A,\,t-1}) C_{t-1}];\quad (1 + A - A' < t \le T)$$

Estimated observations ($1 \le t \le T$)

(M.18) $$\overline{I_{1t}} = q_1(B_t - f_{1t} D_t);\quad (t \in \mathbf{T}_1)$$

(M.19) $$\overline{I_{2t}} = q_2(B_t - f_{2t} D_t);\quad (t \in \mathbf{T}_2)$$

(M.20) $$\overline{p_{at}} = u_{at};\ (1 \le a < A'_t)$$

(M.21) $$\overline{p_{A'_t,t}} = \sum_{a=A'}^{A_t} u_{at}$$

can be computed directly from N_{at}. All quantities produced by these calculations (estimates, underlying states, moments) can be considered functions of the parameter vector Φ.

The algorithm in Table A.2 is completely deterministic. To render it statistical, we impose three sources of error: (*i*) autoregressive lognormal process error among the recruitments R_t, (*ii*) lognormal measurement error in the indices I_{it} ($i = 1, 2$), and (*iii*) multivariate logistic measurement error in the observed proportions p_{at}. Schnute and Richards (1995) show that these sources of error introduce the following additional parameters: (*i*) autoregressive recruitment coefficients R and γ, plus a recruitment standard deviation σ_1, (*ii*) an index standard deviation τ_1, and (*iii*) a standard deviation τ_2 for the multivariate logistic distribution. We make the simplifying assumption that the standard deviation τ_1 applies to both I_{1t} and I_{2t}. We also follow Schnute and Richards (1995) in excluding process error from the dynamics (M.15)–(M.17).

Table A.3 defines the likelihood function $L(\Theta)$ for this stochastic model, where the full parameter vector Θ in (L.1) extends Θ to include the additional parameters R, γ, σ_1, τ_1, and τ_2. To calculate $L(\Theta)$, first obtain the estimates $\overline{p_{at}}$ and $\overline{I_{it}}$ from

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Table A.3. Likelihood $L(\Theta)$ for a stochastic counterpart to the model in Table A.2.

Parameters

(L.1) $\Theta = (\Phi, R, \gamma, \sigma_1, \tau_1, \tau_2)$

(L.2) $\kappa^2 = \sigma_1^2 + \tau_1^2, \quad \rho = \dfrac{\sigma_1^2}{\sigma_1^2 + \tau_1^2}$

(L.3) $\sigma_1^2 = \rho\kappa^2, \quad \tau_1^2 = (1-\rho)\kappa^2$

Residuals

(L.4) $\xi_{2-A'} = \log(R_{2-A'}) - \log(R)$

(L.5) $\xi_{it} = \log(R_t) - (1-\gamma)\log(R) - \gamma\log(R_{t-1}); \ (2 - A' < t \le T)$

(L.6) $\zeta_{1t} = \log(I_{1t}) - \log(\overline{I_{1t}}); \ (t \in \mathbf{T}_1)$

(L.7) $\zeta_{2t} = \log(I_{2t}) - \log(\overline{I_{2t}}); \ (t \in \mathbf{T}_2)$

(L.8) $\eta_{at} = \log(p_{at}) - \log(\overline{p_{at}}) - \dfrac{1}{A_t'}\displaystyle\sum_{a=1}^{A_t'} [\log(p_{at}) - \log(\overline{p_{at}})]$

Likelihoods

(L.9) $L_1(\Theta) = (1-\gamma^2)^{1/2}\,((2\pi)^{1/2}\,\sigma_1)^{2-A'-T} \exp\left[-\dfrac{1}{2\sigma_1^2}\left((1-\gamma^2)\xi_{2-A'}^2 + \displaystyle\sum_{t=3-A'}^{T}\xi_{it}^2\right)\right]$

(L.10) $L_2(\Theta) = \displaystyle\prod_{i=1}^{2}\prod_{t\in\mathbf{T}_i}\left[\dfrac{1}{(2\pi)^{1/2}\tau_1}\exp\left(-\dfrac{1}{2\tau_1^2}\zeta_{it}^2\right)\right]$

(L.11) $L_3(\Theta) = \displaystyle\prod_{t=1}^{T}\left[\dfrac{(A_t')^{1/2}}{((2\pi)^{1/2}\tau_2)^{A_t'-1}}\exp\left(-\dfrac{1}{2\tau_2^2}\sum_{a=1}^{A_t'}\eta_{at}^2\right)\right]$

(L.12) $L(\Theta) = \displaystyle\prod_{i=1}^{3} L_i(\Theta)$

Table A.2 and then proceed sequentially through (L.4)–(L.12). Equations (L.2) and (L.3) allow Θ to be expressed in terms of either (σ_1, τ_1) or (κ, ρ). In the analyses here, we fix ρ and allow κ to vary.

To simplify mathematical notation, we omit two technical details from the calculations in Tables A.2 and A.3. First, missing age data for the years 1986 and 1988 (Fig. 1) require that two years be dropped from the product (L.11). Second, in our calculations, we alter the definition of age-classes to require that $p_{at} \ge 0.02$ for every a and t. We accomplish this by grouping consecutive ages, where necessary, into a single age-class. This grouping reduces the influence on the likelihood of observations (presumably with high measurement error) obtained from only a few fish. In trial runs, we found that grouping produced much smaller residuals overall than those obtained without grouping.

Appendix B. Statistical inference

We estimate parameters and confidence regions from the log likelihood function $\ell(\Theta) = \log L(\Theta)$ computed from (L.12). Tables A.2–A.3 show that all states, moments, and residuals can be computed from the parameter vector Θ. Thus, the maximum likelihood estimate $\hat{\Theta}$ gives corresponding estimates of all quantities of interest within the model. For a somewhat different model, Schnute and Richards (1995, Table 4) show that, given ρ, analytic estimates of κ, σ_1, τ_1, and τ_2 can be obtained. Similar results for the model here can be used to simplify the estimation process.

To estimate uncertainty, we use the Taylor approximation

(B.1) $\ell(\Theta) \approx \ell(\hat{\Theta}) - \dfrac{1}{2}(\Theta - \hat{\Theta})' J(\hat{\Theta})(\Theta - \hat{\Theta})$

where the prime ($'$) symbol in this context denotes transpose and the negative Hessian

(B.2) $J(\hat{\Theta}) = -\left.\dfrac{\partial^2 \ell}{\partial \Theta^2}\right|_{\Theta=\hat{\Theta}}$

is called the "observed Fisher information matrix". The approximation (B.1) contains no linear term in Θ because the gradient $\partial\ell/\partial\Theta$ is 0 when $\Theta = \hat{\Theta}$. From a Bayes perspective, with a diffuse prior on Θ, $L(\Theta)$ is proportional to the posterior $P(\Theta)$. Furthermore, (B.1) is equivalent to a multivariate normal approximation to the Bayes posterior, in which Θ has mean $\hat{\Theta}$ and covariance matrix $J^{-1}(\hat{\Theta})$. This normal approximation also has meaning in a frequentist context (Tanner 1993, chap. 2), and highest posterior density confidence regions correspond exactly to regions obtained by likelihood ratio tests.

If $\Psi(\Theta)$ is a vector of m quantities that depend on the n-dimensional parameter vector Θ, then the Bayes posterior for Ψ is approximately normal with mean $\hat{\Psi} = \Psi(\hat{\Theta})$ and covariance matrix (Tanner 1993, p. 17)

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$$\text{(B.3)}\quad \mathrm{Cov}[\boldsymbol{\Psi}] \approx \left(\left.\frac{\partial \boldsymbol{\Psi}}{\partial \boldsymbol{\Theta}}\right|_{\boldsymbol{\Theta}=\hat{\boldsymbol{\Theta}}} \right)' J^{-1}(\hat{\boldsymbol{\Theta}}) \left.\frac{\partial \boldsymbol{\Psi}}{\partial \boldsymbol{\Theta}}\right|_{\boldsymbol{\Theta}=\hat{\boldsymbol{\Theta}}}$$

where the gradient matrix $\partial\boldsymbol{\Psi}/\partial\boldsymbol{\Theta}$ has dimensions $n \times m$. As with $\boldsymbol{\Theta}$, this result for $\boldsymbol{\Psi}$ can be given a frequentist interpretation.

In the special case $m = 1$, $\boldsymbol{\Psi}$ is a scalar with variance (B.3). When $m = 2$, denote the coordinates of $\boldsymbol{\Psi}$ by x and y; thus, $\boldsymbol{\Psi} = (x, y)$, $\hat{\boldsymbol{\Psi}} = (\hat{x}, \hat{y})$, and

$$\text{(B.4)}\quad \mathrm{Cov}[\boldsymbol{\Psi}] = \begin{pmatrix} \mathrm{Var}[x] & \mathrm{Cov}[x,y] \\ \mathrm{Cov}[x,y] & \mathrm{Var}[y] \end{pmatrix} = \begin{pmatrix} \sigma_x^2 & \rho_{xy}\sigma_x\sigma_y \\ \rho_{xy}\sigma_x\sigma_y & \sigma_y^2 \end{pmatrix}$$

Combining (B.3) and (B.4) gives estimates of two standard errors (σ_x, σ_y) and a correlation (ρ_{xy}) for the pair (x, y). An α-level highest posterior density contour for (x, y) is defined by the ellipse

$$\text{(B.5)}\quad \frac{1}{1-\rho_{xy}^2}\left[\frac{(x-\hat{x})^2}{\sigma_x^2} - \frac{2\rho_{xy}(x-\hat{x})(y-\hat{y})}{\sigma_x\sigma_y} + \frac{(y-\hat{y})^2}{\sigma_y^2}\right] = \chi_\alpha^2(2)$$

where $\chi_\alpha^2(2)$ denotes the α-level for a χ^2 distribution with 2 df. For example, $\chi_{0.95}^2(2) = 5.99$. To plot this contour, compute points (x, y) from a parameter ω ($0 \le \omega \le 2\pi$) as follows:

$$\text{(B.6)}\quad r = \left[\frac{(1-\rho_{xy}^2)\chi_\alpha^2(2)}{\cos^2\omega - 2\rho_{xy}\cos\omega\sin\omega + \sin^2\omega}\right]^{\frac{1}{2}}$$

$$\text{(B.7)}\quad x = \hat{x} + \sigma_x r\cos\omega; \quad y = \hat{y} + \sigma_y r\sin\omega.$$

Confidence contours in Fig. 8 for various years t are computed from (B.6) and (B.7) with $(x, y) = (\log S_{t-7}, \log R_t)$. After transforming from logarithmic to original units for S and R, the elliptical contours become elongated ovals.

Computationally, the algorithms described here require procedures to obtain $\hat{\boldsymbol{\Theta}}$ by maximizing $\ell(\boldsymbol{\Theta})$ and to compute the derivatives in (B.2) and (B.3). The software package AD Model Builder (Otter Research Ltd. 1994) fulfills these requirements by providing a gradient-based search algorithm and facilities for automatic differentiation (Griewank and Corliss 1991) of C++ code. In addition, matrix classes within the software simplify other calculations, such as the matrix arithmetic in (B.3).

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Ultraviolet radiation and photosynthesis by Georgian Bay phytoplankton of varying nutrient and photoadaptive status

J.A. Furgal and R.E.H. Smith

Abstract: The photosynthesis–irradiance responses of phytoplankton from Georgian Bay (Laurentian Great Lakes) were measured in 1993 to determine the influence of ultraviolet-B (UV-B, 290–320 nm) radiation and the interacting effects of natural changes in nutrient status and photoadaptive state. Silicate concentrations and phosphorus availability (inferred from ^{32}P incorporation) varied widely but had little direct influence on photosynthetic performance. UV-B caused up to 80% loss of photosynthetic capacity in 1-day simulated surface exposures (artificial light) and up to 40% loss in half-day exposures (natural light), quantitatively consistent with reported responses of marine phytoplankton. The widely varying nutrient status and photoadaptive state (as inferred from photosynthetic light responses) of the phytoplankton had no significant influence on their sensitivity to UV-B radiation, and midsummer communities were as sensitive as spring or fall communities. To a first approximation, the impact of UV-B was proportional to the seasonal variation in surface UV-B irradiance being maximal near the summer solstice.

Résumé : Les réponses photosynthèse-éclairement énergétique du phytoplancton de la baie Géorgienne (Grands Lacs laurentiens) ont été mesurées en 1993 pour déterminer l'effet du rayonnement ultraviolet B (UV-B, 290–320 nm) et les effets interagissant des changements naturels sur l'état nutritionnel et l'état de photoadaptation. Les concentrations de silicate et la disponibilité du phosphore (déduit à partir de l'incorporation de ^{32}P) ont varié considérablement, mais n'avaient peu d'effet direct sur le rendement photosynthétique. Les UV-B ont causé une perte de la capacité photosynthétique allant jusqu'à 80 % dans des expositions de surface simulées d'un jour (lumière artificielle) et jusqu'à 40 % dans des expositions d'une demi-journée (lumière qui correspond du point de vue quantitatifréponses signalées dans le cas du phytoplancton marin. L'état nutritionnel et l'état de photoadaptation (tel que déduit à partir des réponses à la lumière photosynthétique) fortement variable du phytoplancton n'avaient pas d'effets significatifs sur la sensibilité de ce dernier au rayonnement UV-B, et les communautés du milieu de l'été étaient aussi sensibles que les communautés du printemps ou de l'automne. En première approximation, les effets des UV-B étaient proportionnels à la variation saisonnière de l'éclairement énergétique de la surface par les UV-B, atteignant son maximum près du solstice d'été.
[Traduit par la Rédaction]

Introduction

The negative effects of ultraviolet radiation (UVR) on phytoplankton photosynthesis, recognized many years ago (Steeman Neilsen 1964; Rodhe et al. 1966), have attracted more attention recently as UV-B (280–320 nm) radiation levels at ground level increase in response to continuing stratospheric ozone depletion (Kerr and McElroy 1993; Madronich et al. 1995). Modern measurements show that significantly damaging quanitities of UV-B can penetrate through the upper third of the photic zone in clear marine systems (e.g., Smith and Baker 1989; Smith et al. 1992) and some freshwater lakes (Gala and Giesy 1991; Scully and Lean 1994). The vernal destruction of stratospheric ozone in Antarctic polar regions causes springtime fluxes of UV-B at ground level to reach levels normally observed only in midsummer (Madronich et al. 1995). In north temperate regions, the vernal destruction of stratospheric ozone is less pronounced and surface fluxes of UV-B still follow seasonal trends similar to photosynthetically active radiation (PAR), with a maximum near the summer solstice (Kerr and McElroy 1993). Thus, late-spring and early summer phytoplankton in many north-temperate lakes can be exposed to high fluxes of potentially damaging UV-B.

To date, few studies have attempted to assess the loss of production due to UVR in natural freshwater phytoplankton communities (Gala and Giesy 1991; Milot-Roy and Vincent 1994; Moeller 1994). In Lake Michigan, a large and relatively transparent lake, up to 72% of potential primary production in near-surface samples was lost owing to ambient levels of UVR, while the average loss over the entire photic zone was 13% for the May–August period at current UVR levels (Gala and Giesy 1991). Integrated losses for clear-water systems such as the oligotrophic upper Great Lakes may be even more severe than estimated so far for Lake Michigan, depending on the kinetics of UVR damage and recovery (Cullen and Lesser 1991; Lesser et al. 1994), the nature of the stratification regime (e.g., Milot-Roy and Vincent 1994), and the influence of factors that modify the sensitivity of phytoplankton to UV-B.

In Lake Michigan, midsummer phytoplankton suffered less inhibition by UV-B than spring or fall communities, despite high incident radiation, suggesting that the species composition or the relatively high light adapted state of the summer

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phytoplankton conferred some resistance (Gala and Giesy 1991). The physiology of UV-B photoinhibition has much in common with inhibition by PAR, and shade-adapted plants are expected to be relatively vulnerable to both PAR- and UVR-dependent photoinhibition (Greenberg et al. 1989; Vincent and Roy 1993; Tevini 1994). Shade-adapted phytoplankton from below the pycnocline in the South Pacific Ocean have indeed been shown to be more sensitive to UV-B than their surface-dwelling contemporaries (Behrenfeld et al. 1993*b*). Nutrient status may also influence algal sensitivity to UV-B, possibly because protein turnover essential to recovery processes is inhibited when nitrogen, and likely other inorganic nutrients, are limiting (Cullen and Lesser 1991; Lesser et al. 1994; but cf. Behrenfeld et al. 1994). There are as yet no reports of field tests for the effects of variable nutrient status on UV-B impact, although nutrient limitation has been blamed for the inability of some freshwater phytoplankton to develop UV-B resistance despite high chronic exposure (Moeller 1994).

The influence of nutrient status on photosynthetic performance even without the complicating effects of UV-B is poorly defined. The correlation of photosynthetic capacity and efficiency with nutrients may be poor or nonexistent in some communities (e.g., Côté and Platt 1983) and strong in others (Welschmeyer and Lorenzen 1981; Fee et al. 1987). The apparently inconsistent effects of nutrients on photosynthesis undoubtedly reflects at least in part the important and confounding influences of other factors such as species composition, light climate, and temperature (Harris 1978; Platt and Jassby 1976; Senft 1978; Côté and Platt 1983; Sephton and Harris 1984; Babin et al. 1995) but may also reflect the difficulty of quantifying nutrient status in some communities, particularly those in which P is the most limiting nutrient. It has been known for many years that in P-limited systems standard chemical assays for "orthophosphate" are unreliable as a measure of either the true available P concentration or the nutrient status of the phytoplankton (Lean and Pick 1981; Lean et al. 1987). There are alternative methods that use the physiology of the algae themselves to quantify P status. The phosphorus deficiency index (PDI) of Lean and Pick (1981) assesses the P status of phytoplankton by comparing the capacities for photosynthetic carbon and P uptake, thus avoiding the analytical problems of measuring orthophosphate at limiting concentrations. Although apparently well suited to the task of assessing nutrient status in many freshwater phytoplankton communities, including those of the Great Lakes, the PDI and related methods have not yet been used to test effects of nutrients on photosynthesis or its sensitivity to UV-B.

In the present study, we used experiments under both artificial and natural light to test the sensitivity to UV-B of phytoplankton from a site in the clear and oligotrophic upper Great Lakes, and to determine the modifying influences of nutrient status and photoadaptive state. Previous work in the Great Lakes (Gala and Giesy 1991) has not clearly separated the effects of UV-B, which is sensitive to stratospheric ozone depletion, from those of UV-A (320–400 nm), which is not sensitive to ozone depletion and has both beneficial and deleterious effects on microalgae and other plants (Kirk 1983; Smith et al. 1992; Vincent and Roy 1993; Quesada et al. 1995). The work reported here was designed explicitly to isolate the effects of UV-B and determine whether the magnitude and seasonal trends of UVR inhibition reported by Gala and Giesy (1991) for Lake Michigan are also true for UV-B effects in a comparable freshwater system (Georgian Bay).

Materials and methods

Sampling and incubation protocols

The study site (44°50′N, 81°02′W) was situated on Colpoys Bay, southwestern Georgian Bay, approximately 300 m offshore in 65 m of water. Sampling was done just before dawn once every 3 weeks from May 20 to November 4, 1993, for a total of nine sampling dates. On each occasion, the thermal structure of the water column was measured using a SeaCat SBE 19-3 profiler, and epilimnetic depth determined. Mid-epilimnetic samples were then collected using a 5-L Van Dorn sampler, transferred to acid-cleaned polycarbonate bottles, and transported in a cooler to the isotope laboratory at the University of Waterloo. If thermal stratification was too weak to define the epilimnion clearly, samples were taken at 5 m. Experiments began within 3 h of sampling.

Phytoplankton samples were exposed to either simulated natural illumination in an indoor incubator (artificial light experiments) or natural illumination out of doors (natural light experiments). The artificial light experiments were designed to simulate a full-day, midsummer exposure near the surface and offered a standard, reproducible treatment to detect seasonal variations in sensitivity. The natural light experiments, which for logistical reasons could encompass only half a day's UVR exposure, acted as a reference to assess phytoplankton response under the natural, variable spectrum. In comparing phytoplankton responses among experiments and with the literature, we compensated for variations in spectral quality by applying the biological weighting function (BWF) of Behrenfeld et al. (1993*a*):

$$(1) \qquad \varepsilon(\lambda) = 3.318 \times 10^{17}\, e^{-0.13\,448\,\lambda}$$

where $\varepsilon(\lambda)$ is the wavelength-dependent efficiency of photoinhibition and λ is wavelength (290–347 nm). Although other, more rigorously defined, BWFs exist for phytoplankton photosynthesis (Cullen et al. 1992) the general form of the alternative BWFs is similar. Furthermore, doses calculated using eq. 1 can be used to predict the UV-dependent loss of photosynthesis in natural marine phytoplankton communities by the empirical relationship (Behrenfeld et al. 1993*a*):

$$(2) \qquad \%\text{inhibition} = 0.007 Q_{\text{exp}}$$

where Q_{exp} is the dose, weighted according to eq. 1. We used eq. 2 to calculate the percent inhibition expected in our experiments to determine whether the freshwater phytoplankton of Georgian Bay responded similarly to the varied marine communities studied by Behrenfeld et al. (1993*a*).

Artificial light experiments were done with polystyrene tissue culture flasks in a controlled environment chamber for 6 h at in situ temperatures. Photosynthetically active radiation (PAR; 400–700 nm), ultraviolet A (UV-A; 320–400 nm), and ultraviolet B (UV-B; 280–320 nm) radiation were provided using cool white fluorescent (General Electric F24T12), UV-A (RPR 3500 UVA lamp, Southern New England Ultraviolet Company, Conn.), and UV-B (RPR 3000 UVB lamp) light sources, respectively. Neutral-density filters and a UV-B selective filter (plate glass) were used to vary PAR from 30 to 600 μmol photons·m^{-2}·s^{-1} (plus a dark control) with roughly proportional amounts of UV-A (Table 1) as estimated from optical data for Lake Michigan in Gala and Giesy (1991). Incident PAR was measured with a LI-COR spherical quantum sensor and ultraviolet radiation was measured with a Photodyne Model 88XLC radiometer/photometer and 10-nm-wavelength interference filters. The polystyrene flasks effectively removed all UV <280 nm wavelength, and the light reaching the phytoplankton (Fig. 1) was calculated using the measured transmission spectrum of the polystyrene (which did not change with continued exposure to UV-B). One half of the highest PAR group was exposed to UV-B at the realistic ratio of approximately 0.3% UV-B:PAR (Table 1). The unweighted exposure to UV-B

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Table 1. Summary of PAR, UV-A, and UV-B irradiance (μmol photons·m^{-2}·s^{-1}) in each of the six light treatments.

Radiation regime	PAR	UV-A	UV-B
Dark	0.0	0.0	0.00
1	30.0	0.1	0.00
2	60.0	0.2	0.00
3	150.0	0.6	0.00
4	600.0	4.5	0.02
5	600.0	5.6	1.63

approximated the midday summer regime under clear skies at approximately 2.5 m depth in the clear waters of offshore Lake Michigan (Gala and Giesy 1991). The daily dose weighted according to eq. 1 was 7015 J·m^{-2}, representative more of the 0.5–1.0 m depth interval in Lake Michigan.

In natural light experiments, phytoplankton were exposed to solar radiation in UVT Plexiglas containers (same dimensions as tissue culture flasks) for 6 h at in situ temperatures regulated by a water bath. The experiment consisted of two treatments receiving solar radiation attenuated with neutral-density screens to 50% of incident to simulate the UV-B received at depths of about 1 m in Lake Michigan (Gala and Giesy 1991). One treatment received the full natural spectrum, and a second (UV-B removed) used the same type of UV-B selective filter as used under artificial light (plate glass) to eliminate essentially all wavelengths from 280 to 320 nm. With the exception of June 10, 1993, natural light experiments started between 13:00 and 13:30 each day, roughly corresponding with solar noon when UV-B flux rates are at their highest. The experiment on June 10 started at 14:30. All experiments except on September 2 (a rainy day with continous overcast) took place under clear or nearly clear skies.

Incident spectral radiation during the natural light experiments was estimated from measurements at the Downsview, Ont., station of the Atmospheric Environment Service (AES), Environment Canada, approximately 80 km east of our experimental site, that were kindly made available by Environment Canada scientists. Weather conditions did not differ substantially between the two locations except as noted in the Discussion. The AES radiation records provided integrated measurements at wavelength intervals of 0.5 nm at approximately 1-h intervals, collected by a Brewer radiometer (Kerr and McElroy 1993). We combined the incident radiation data with the measured transmission characteristics of our incubation materials to calculate the integrated spectral radiation input (Table 2).

Photosynthesis measurements

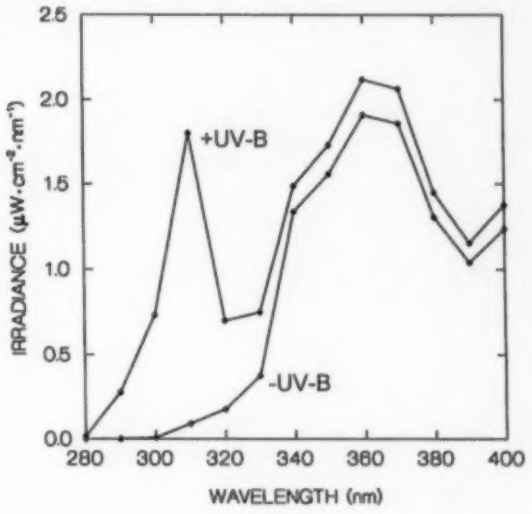
To measure photosynthetic carbon assimilation, water was spiked with ^{14}C-labeled bicarbonate (ICN Biomedicals; 6.7×10^{11} Bq/mL) and subsampled for total initial activity using liquid scintillation spectrometry (LSC). Subsamples of 5 mL were also filtered onto 25-mm Whatman GF/F glass fibre filters (the standard filters used here) as time zero controls for uptake of ^{14}C. The remaining solution was distributed in 60-mL aliquots among the incubation vessels, which were randomly assigned (in triplicate) to the different light treatments. At the end of the incubation (6 h), 5 mL from each flask was filtered for determination of total carbon assimilation. All filters were acidified to drive off unincorporated $^{14}CO_2$ before adding 5 mL of Ecolume scintillant and analyzing by liquid scintillation spectroscopy.

Roughly 200 mL of lake water from sealed polycarbonate bottles was used for triplicate alkalinity determinations using a simplified Gran titration (American Public Health Association et al. 1989). Dissolved inorganic carbon (DIC) was determined from the $CaCO_3$ alkalinity and used to calculate carbon uptake from the ^{14}C results after correcting for uptake in the dark controls.

To characterize photosynthesis–light relationships, photosynthetic carbon uptake rates were fitted to the model (Platt et al. 1982):

$$P = P_m(1 - e^{(-\alpha I)}) \quad (3)$$

Fig. 1. Spectral irradiance in +UV-B and –UV-B treatments under artificial light, corrected for transmission through the incubation containers.



where photosynthetic rates are normalized to chlorophyll *a* and $a = \alpha/P_m$. P_m is the maximum light saturated rate of photosynthesis, a measure of photosynthetic capacity. The parameter α is the initial slope of the photosynthesis–irradiance (*P–I*) curve at limiting irradiance and is a measure of light utilization efficiency. The model was fitted using nonlinear least squares regression (Wilkinson 1990). The number of different irradiances in our design was minimal for this purpose, but we were able to estimate α and P_m with good statistical precision in all cases (Table 2). On two of the nine experimental dates, there appeared to be significant photoinhibition at the highest PAR level, but we were unable to fit more sophisticated models that included a photoinhibition term, probably because of our limited number of irradiance treatments. The implications for our conclusions will be considered below.

Nutrient and light status

The photoadaptation parameter, I_k (μmol photons·m^{-2}·s^{-1}), was calculated as the ratio of P_m to α. Together with α, I_k provides a measure of the photoadaptive state of the phytoplankton in that lower I_k values often reflect adaptation to more shaded conditions (see Discussion)

The phosphorus deficiency index (PDI) of Lean and Pick (1981) was calculated as the ratio of P_m to V_{max}, where V_{max} is the maximum (substrate-saturated) P-uptake rate. Low values of the PDI (0–10) denote extreme P deficiency, medium values (10–100) indicate moderate to low deficiency, while values >100 denote P sufficiency (Lean and Pick 1981). V_{max} was determined from radiophosphorus ($^{32}PO_4$) uptake in the presence of saturating concentrations of phosphate (50 μg P·L^{-1} as KH_2PO_4). Phosphorus uptake was measured in triplicate using ^{32}P-H_2PO_4 retention on Whatman GF/F filters after incubation in the dark at in situ temperature for 1 h.

Samples for soluble reactive phosphorus (SRP) and soluble reactive silicate analysis were filtered through 0.4-μm pore size polycarbonate filters. SRP was measured with the molybdate–ascorbate assay, and silicate concentration with the molybdate method (Strickland and Parsons 1972).

Samples of 100 mL for chlorophyll *a* determination were filtered onto 25-mm Whatman GF/F glass fibre filters, which were extracted for 18–24 h in 10 mL of 9:1 acetone:water at –20°C. Chlorophyll *a* concentrations were then determined fluorometrically using a Turner

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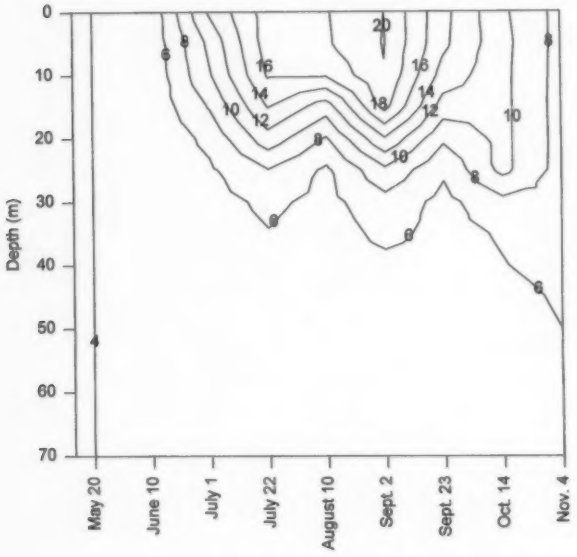
Fig. 2. Depth–time plot of temperatures (°C) at the sampling site in Colpoys Bay, Georgian Bay, 1993.



Designs model 10AU fluorometer calibrated against pure chlorophyll *a* (Smith and Maly 1993).

Results

Seasonal variation of environmental conditions

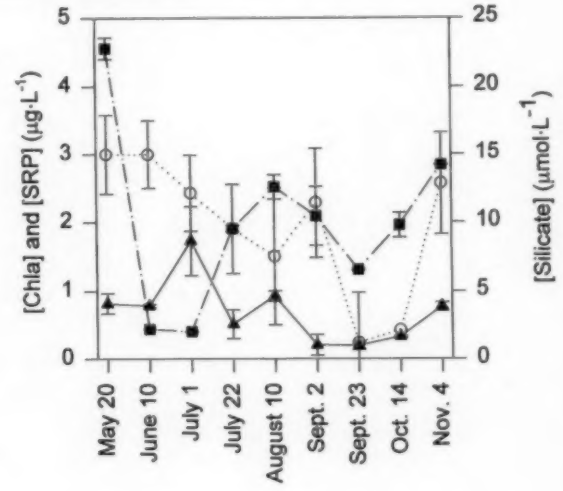
Thermal stratification began to form in late June and was well established by late July (Fig. 2). The bay remained stratified throughout the rest of the summer until mid-October, when the thermocline deepened and the water column once again became isothermal. The depth of the epilimnion varied from 5 m in early July to between 10 and 15 m throughout the rest of the summer and into late September.

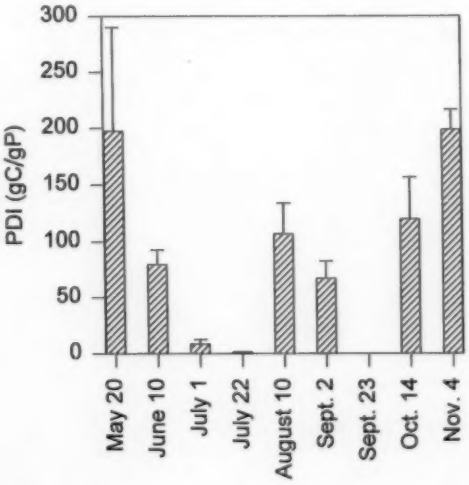
Surface water temperatures increased from 4.0°C on May 20, to a maximum of 20.0°C on September 2, and then slowly decreased to 4.9°C on November 4. Hypolimnetic water temperature in Colpoys Bay varied between a minimum temperature of 4.0°C on May 20 and a maximum of 5.0°C on August 12.

Epilimnetic chlorophyll *a* (Chl *a*) concentrations varied between a minimum of 0.19 $\mu g \cdot L^{-1}$ on September 23 and a maximum of 1.73 $\mu g \cdot L^{-1}$ on July 1 (Fig. 3A). The general trend was for relatively high Chl *a* concentrations on the first three sampling dates when stratification was weak or newly established, with values declining thereafter to the early autumn minimum. The peak in Chl *a* concentration appeared to coincide with the onset of strong summertime stratification. Chl *a* concentrations increased in the fall as stratification weakened but did not attain values as high as those in early summer.

Soluble reactive phosphorus (SRP) concentration varied from a maximum of 3.0 $\mu mol \cdot L^{-1}$ on May 20 to a minimum of 0.2 $\mu mol \cdot L^{-1}$ on September 23 (Fig. 3A). SRP concentrations decreased from late spring (May 20) to early autumn

Fig. 3. Nutrient and chlorophyll *a* time series at the sampling site in Colpoys Bay, showing mean and 95% CI for each date. (A) Chlorophyll *a* (▲), soluble reactive silicate (■), and soluble reactive phosphorus (SRP; ○) concentrations. (B) Phosphorus deficiency index (PDI).





(September 23), except for a relatively high concentration on September 2, and then increased again in October–November. Reactive silicate concentrations were at their maximum at the beginning of the sampling season, with a value of 23.0 $\mu mol \cdot L^{-1}$ on May 20 (Fig. 3A). The early maximum was followed by a marked decrease and a minimum of 2.5 $\mu mol \cdot L^{-1}$ on July 1, coinciding with the maximum Chl *a* concentration. Silicate concentrations increased subsequently to a summertime peak on August 10. Concentrations then decreased to another minimum on September 23 before increasing again in October–November.

The phosphorus deficiency index (PDI) decreased from the maximum observed value of 197.2 on May 20 to the minimum observed value of 1.31, corresponding to extreme deficiency,

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on July 22 (Fig. 3B). The PDI rose steeply from July 22 to August 10. PDI was not measured on September 23 so we do not know if there may have been an early autumn minimum similar to those observed for SRP, silicate, and Chl *a* concentrations. PDI values did rise in October–November, however, similar to the other nutrient measures.

Photosynthetic carbon assimilation

On June 10, during near-isothermal conditions, the highest carbon assimilation rate (1.069 mg C·mg Chl $a^{-1}\cdot h^{-1}$) was observed in the second-highest PAR treatment and a lower rate (0.878 mg C·mg Chl $a^{-1}\cdot h^{-1}$) in the higher PAR treatment (Fig. 4), indicating photoinhibition of 18% or more at 600 µmol photons·$m^{-2}\cdot s^{-1}$ relative to the optimum. On November 4, when the water column was again very weakly stratified, the highest PAR treatment resulted in photoinhibition and an assimilation rate 80% of that at the optimum observed in the second-highest PAR treatment. All other dates showed a maximum carbon assimilation rate in the highest PAR treatment (e.g. August 10, Fig. 4) without UV-B exposure and thus revealed no inhibition by PAR.

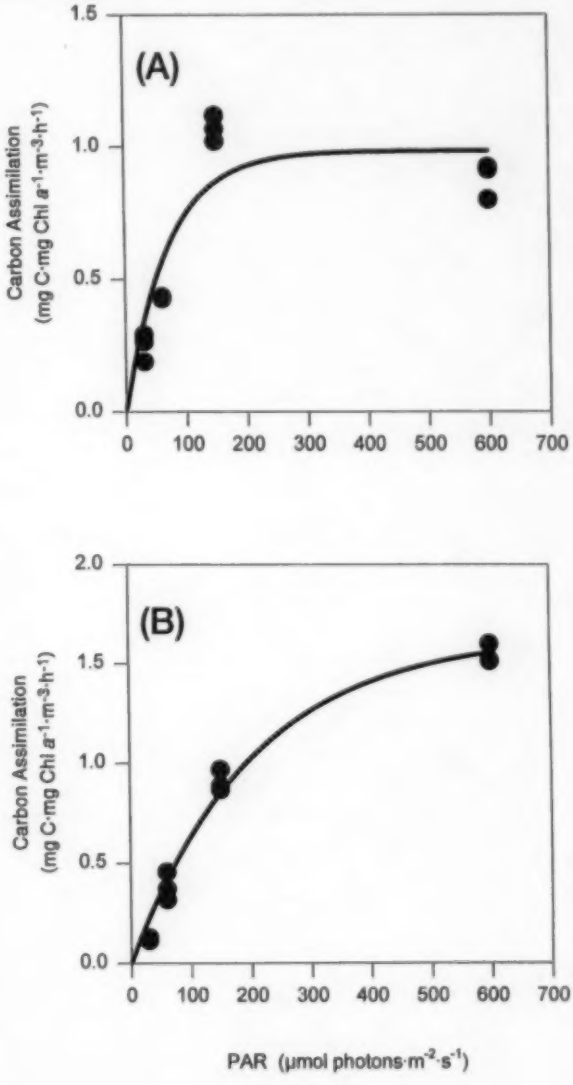
The response of Chl *a* normalized carbon assimilation rate to PAR irradiance was adequately described by the chosen function (eq. 3) in all cases, with adjusted r^2 values ranging from 0.59 to 0.98 and averaging 0.86. The fitted P_m values for Colpoys Bay ranged from a minimum of 0.68 mg C·mg Chl $a^{-1}\cdot h^{-1}$ on May 20 to a maximum of 5.90 mg C·mg Chl $a^{-1}\cdot h^{-1}$ on September 23, with an average value of 2.19 mg C·mg Chl $a^{-1}\cdot h^{-1}$ (Table 2). The trend among sampling dates was for minimal values on the earliest and latest sampling dates with maxima in early autumn (September). For the two dates displaying photoinhibition by PAR the fitted P_m values were underestimates but only by about 9%. There was in fact no significant difference between fitted values of P_m and the maximum observed assimilation rate for any of the sampling dates according to *t* tests ($p > 0.05$).

The fitted values for α ranged from a minimum of 0.004 on July 22 to a maximum of 0.034 on September 23, with a mean value of 0.016 mg C·mg Chl $a^{-1}\cdot h^{-1}\cdot(\mu mol\cdot m^{-2}\cdot s^{-1})^{-1}$ (Table 2). α was low throughout the summer months (July–August) and higher in the autumn. As for the maximum assimilation rate, the fitted values of α will be underestimates for the two dates with photoinhibition, but the bias is small.

I_k ranged from 70 and 65 µmol·$m^{-2}\cdot s^{-1}$, for isothermal conditions on June 10 and November 4, respectively, to a high of 402 µmol·$m^{-2}\cdot s^{-1}$ on July 22, under well-established stratification (Table 2). I_k declined thereafter even though stratification continued strong, perhaps reflecting the waning daily solar irradiance with the approach of the autumnal equinox.

Regression analysis of linear relationships between the fitted and derived photosynthetic parameters (P_m, I_k, and α) and measured environmental factors (Chl *a* concentration, water temperature, daylength, SRP concentration, silicate concentration, and PDI) showed a significant negative relationship between α and daylength ($r^2 = 0.458$, $p = 0.045$) and Chl *a* ($r^2 = 0.466$, $p = 0.043$), a negative relationship between P_m and Chl *a* concentration ($r^2 = 0.576$, $p = 0.018$) and SRP ($r^2 = 0.640$, $p = 0.017$), and a significant positive relationship between I_k and water temperature ($r^2 = 0.491$, $p = 0.036$). No other statistically significant bivariate relationships were found. Multiple regressions on temperature in combination with each of the nutrient measures in turn also failed to reveal significant linear multivariate relationships. Examination of scatterplots did not suggest any significant nonlinear relationships that might have been missed by the linear regression analyses.

Fig. 4. Photosynthesis–irradiance responses showing observed rates and the fitted value of the model (eq. 3) for (A) June 10 and (B) August 10.



Effects of ultraviolet radiation

In artificial light experiments, carbon assimilation rates were always lower in the +UV-B than the –UV-B treatment (Table 2). The UV-B inhibition ranged from a minimum of 41% on May 20 to a maximum of 82% on July 22 (Table 2). The lower value (41%) corresponded to one experimental date (May 20) on which the confidence interval for percent inhibition was unusually broad; the range among the eight other

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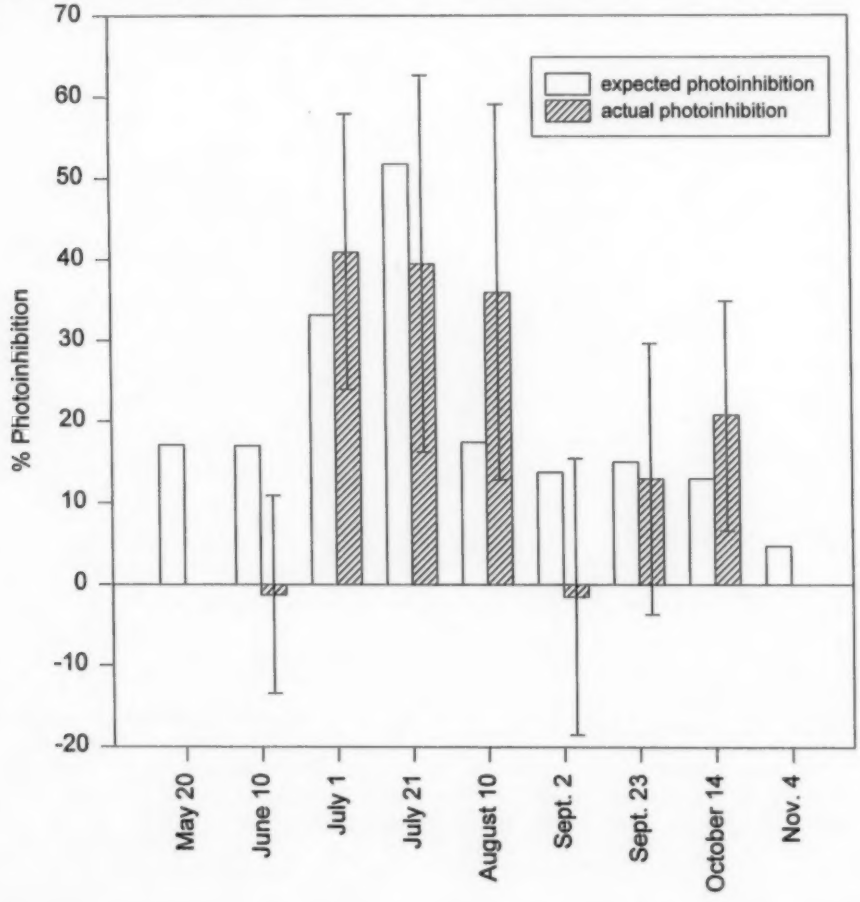
Table 2. Photosynthetic parameters (with 95% C.I), UV-B inhibition, and incident radiation data for outdoor experiments for phytoplankton in Colpoys Bay, 1993.

Date	α	P_m	I_k	UV-B inhibition (%)		Outdoor incident radiation ($kJ \cdot m^{-2}$)	
				Indoor	Outdoor	Unweighted	Weighted
May 20	0.008±0.001	0.676±0.05	85±3.2	40.9±29.8	na	12.48	1.48
June 10	0.014±0.006	0.984±0.179	70±32.7	63.0±6.03	3.3±45.1	11.15	1.47
July 1	0.005±0.004	0.749±0.345	150±138.3	53.3±13.4	40.0±62.2	20.90	2.86
July 22	0.004±0.002	1.607±0.772	402±278.6	81.9±17.6	39.5±23.2	36.36	4.46
Aug. 10	0.008±0.001	1.643±0.150	205±31.3	65.2±33.2	36.0±23.1	11.36	1.50
Sept. 2	0.026±0.004	4.427±0.353	170±29.5	71.4±9.20	−1.5±36.4	9.80	1.19
Sept. 23	0.034±0.010	5.898±0.825	173±56.5	79.2±24.5	13.0±16.6	10.27	1.30
Oct. 4	0.019±0.004	na	na	73.9±8.90	20.8±14.2	10.50	1.12
Nov. 4	0.021±0.007	1.364±0.191	65±23.5	80.8±8.14	na	4.14	0.41

Note: Units for α, P_m, and I_k are mg C·mg Chl $a^{-1} \cdot h^{-1} \cdot (\mu mol\ photons \cdot m^{-2} \cdot s^{-1})^{-1}$, mg C·mg Chl $a^{-1} \cdot h^{-1}$, and $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$, respectively. na, not available.

Fig. 5. Observed and predicted UV-B dependent photoinhibition (with 95% CI for the observed) for natural light experiments.



dates was narrower, from 53 to 82%. By comparison, the inhibition predicted from eq. 2 for the constant dose of 7015 $J \cdot m^{-2}$ was 81%. ANOVA and t tests confirmed that the observed inhibition was significantly greater than zero ($p < 0.05$) on every date. Except for July 22 there was a weak trend towards increasing UV-B inhibition from early summer through autumn, but ANOVA indicated the variation of inhibition among sampling dates was not significant ($p > 0.05$). Correlation analysis failed to reveal any significant linear relationships between the observed inhibition and any of the nutrient

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measures or I_k. The midsummer near-surface conditions simulated in the artificial light experiments thus produced strong and relatively consistent UV-B dependent inhibition of photosynthetic carbon assimilation.

In natural light experiments, carbon assimilation rates were always lower in the +UV-B than the –UV-B treatment (Table 2), with the exception of June 10 (when incubations did not commence until 14:30) and September 2 (a rainy day). UV-B doses varied according to the season and weather (Table 2). The UV-B inhibition ranged from a minimum of –3% on June 10 to a maximum of 40% on July 2 (Fig. 5). There was a strong trend towards increased inhibition during early to midsummer (July 1 – August 10) sampling dates, when the highest doses were observed. The strength of UV-B inhibition predicted from eq. 2 also varied strongly through the observation period, with maximum inhibition predicted to occur on sunny days near midsummer (Fig. 5). The predicted inhibition was not significantly different from the observed for any date with the exception of June 10 (late starting time). Inhibition in the natural light experiments was overall much less than in the artificial light experiments, reflecting the shorter duration of the natural light experiments and the fact that the late summer and autumn experiments were performed under incident radiation considerably less intense than the midsummer conditions simulated in the artificial light experiments.

Discussion

Our results confirmed that Colpoys Bay is a highly oligotrophic system, typical of the upper Great Lakes. Chlorophyll *a* concentrations rarely rose above 1.0 $\mu g \cdot L^{-1}$ throughout the entire season, as seen previously in Colpoys Bay (Smith and Maly 1993) and Georgian Bay proper (Munawar and Munawar 1986). Soluble reactive phosphorus concentrations were generally low while PDI values indicated periods of extreme phosphorus deficiency (Lean and Pick 1981). Silicic acid and PDI results were consistent with depletion of available Si during onset of stratification followed by severe P deficiency during the first few weeks of stratification. Silicic acid concentrations subsequently recovered while the PDI indicated only moderate or slight P limitation for the balance of the observation period. Such a pattern is thought to be a common feature of many oligo-mesotrophic dimictic lakes, including the upper Great Lakes (Fahnenstiel and Scavia 1987; Fahnenstiel et al. 1989), and might be expected to influence phytoplankton physiology, including sensitivity to UV-B, profoundly.

Seasonal variability in photosynthetic physiology, as reflected in photosynthesis–light responses, was certainly appreciable. The range of values and seasonal pattern of variation for P_m resembled those seen in Lakes Michigan, Huron and Ontario (Lean et al. 1987; Fahnenstiel et al. 1989), where maximum P_m values were seen in middle to late stratification period, when nutrient concentrations were low and temperatures high. This would suggest that temperature and nutrient indices should be strongly correlated with P_m, but our regression analyses largely failed to reveal such relationships. There was no statistical evidence that P_m increased as Si and P availability increased, even when we controlled for temperature effects by using multiple regression. Indeed, P_m varied inversely with SRP. It may seem contradictory that the demonstrable nutrient stress in Colpoys Bay did not cause significant lowering of P_m values, but it is a documented characteristic of phyto-plankton that they can continue to synthesize and store organic carbon even when severely limited by lack of nutrients such as phosphorus and nitrogen (Morris 1981). Chlorophyll *a*, to which we normalized the measured photosynthetic rates, is itself a variable aspect of cell composition. In addition, abiotic factors such as temperature and nutrient availability may drive changes in species composition of the phytoplankton, which are then the final determinants of photosynthetic performance (Côté and Platt 1983). The significant correlation we observed between P_m and Chl *a* may actually reflect underlying changes in community composition. In any case, it appears that, even when relatively sophisticated indices of nutrient limitation (the PDI) are used, nutrients explain very little of the variation in photosynthetic capacity (P_m) of the phytoplankton.

The light utilization efficiency (α) of the phytoplankton was also poorly correlated with environmental variables. The lowest values coincided with the most severe P limitation and intermediate temperatures, but temperature and nutrient measurements were uncorrelated with α. The observed inverse correlation with daylength, and the occurrence of lowest values under stratified conditions in early summer, would suggest instead that α was responding to the daily light climate (Platt et al. 1982). The seasonal pattern of I_k also suggested that the photoadaptive state of the phytoplankton varied through the observation period. The range of values and the seasonal patterns resembled those previously at other sites in the Great Lakes, with phytoplankton from the spring and fall mixing periods exhibiting lower I_k values than those under summer stratification (Glooschenko et al. 1974; Fahnenstiel et al. 1989). Thus, our study provides a series of samples representing phytoplankton of varying photoadaptive state as well as nutrient status.

Despite the apparently major variations in their nutrient and light status, the phytoplankton displayed a strong and statistically invariant sensitivity to UV-B under the controlled conditions of the artificial light experiments. This would appear to contradict previous work in Lake Michigan (Gala and Giesy 1991) that indicated much less sensitivity in summer (late July through August) than in spring or fall, and to refute suggestions that photoadaptive state (Gala and Giesy 1991; Behrenfeld et al. 1993*b*) and nutrient status (Dohler 1985; Cullen and Lesser 1991; Lesser et al. 1994) are important modifiers of sensitivity to UV-B. It is possible that more extreme variations in photoadaptive state and nutrient status would reveal more effect, but the ranges of I_k and the PDI were about as wide as could be expected for epilimnetic phytoplankton in the upper Great Lakes. However, allowance must be made for the possible influence of interspecific differences in sensitivity and for the fact that our artificial light sources, like all such systems used to date, did not produce a fully natural spectrum.

One possibility is that the artificial lights produced radiation of such intensity and spectral distribution that any possible variations in sensitivity to natural radiation were overwhelmed. This seems unlikely insofar as the weighted doses, calculated according to eq. 1, were within the natural range previously reported for temperate marine systems (Behrenfeld et al. 1993*a*). The natural light experiments, at much lower doses, gave responses consistent with those in the artificial light experiments, supporting the assumption that eq. 1 is a

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reasonably accurate weighting function for Georgian Bay phytoplankton. In the natural light experiments, the efficiency of inhibition (percent inhibition per unit unweighted dose of UV-B) was 1.9 ± 0.6 $kJ \cdot m^{-2}$ (mean ± 95% CI), not significantly different from the value of 1.5 $kJ \cdot m^{-2}$ reported for summer communities in Lake Michigan by Gala and Giesy (1991) but considerably lower than their values of 3.6 and 2.9 $kJ \cdot m^{-2}$ for spring and fall communities, respectively. Thus, there was no evidence for unusually high sensitivity in either the artificial or natural light experiments. The major difference between our results and those of Gala and Giesy (1991) was instead the relatively low sensitivity per kilojoule of radiation in our spring and fall communities.

Algal species vary considerably in their sensitivity to UV-B (e.g., Karentz et al. 1991; Helbling et al. 1992), and it may be that the spring and fall phytoplankton of Colpoys Bay include fewer sensitive species than in Lake Michigan. We lack phytoplankton enumerations for the year of this study, but in two previous years the phytoplankton of Colpoys Bay were dominated by relatively large diatoms (*Asterionella* and *Fragilaria* in particular) in spring and early summer, succeeding in summer to a mixed community of diatoms, chrysophyceans, and green algae with *Dinobryon* and *Fragilaria* prominent among the larger phytoplankton and *Chromulina* abundant among the smaller (Maly 1992; Smith and Maly 1993). By comparison, Lake Michigan succeeded from a spring dominance by diatoms and chrysophyceans to a summer and fall dominance by blue-green algae (particularly *Microcystis* and *Anabaena*), with the diatom–chrysophycean assemblage apparently more sensitive than the blue-greens (Gala and Giesy 1991). At this coarse level of analysis, taxonomic influences would therefore not appear to explain the differing seasonal patterns of sensitivity and would suggest instead that the phytoplankton in Colpoys Bay should always be more sensitive than in Lake Michigan. Nonetheless, the implications of the considerable differences in taxonomic composition between the lakes deserve closer attention in future studies.

Another potential source of discrepancy between our results for Colpoys Bay and those for Lake Michigan is that we measured the effect of removing only the UV-B (plus a small portion of the UV-A in the 320–330 nm range), while Gala and Giesy (1991) measured the effect of removing not only UV-B but also most of the UV-A. The implications of this difference are hard to evaluate, because UV-A has both beneficial and harmful effects on algae and other plants (Mitchell and Karentz 1993; Quesada et al. 1995) and the interactions between wavebands at varying total irradiance are poorly characterized at present. However, field studies usually indicate that the net effect of UV-A on phytoplankton photosynthesis is negative (e.g., Helbling et al. 1992; Holm-Hansen et al. 1993), and some laboratory studies of algal cultures have given similar results (e.g., Cullen et al. 1992). The efficiency of inhibition in our experiments was equal to or less than that in Gala and Giesy's (1991) experiments, even though we manipulated only the relatively more damaging UV-B, which may suggest that the UV-A in our controls had a strong inhibitory effect in itself. In any case, neither the efficiency of inhibition nor the absolute degree of inhibition followed the seasonal course reported by Gala and Giesy (1991) when the effects of UV-B were isolated as they were in the present study.

Despite the potential for species-specific and environmental influences on algal sensitivity to UV-B, a variety of marine phytoplankton communities have been shown to follow a common response (Behrenfeld et al. 1993*a*) as embodied in eq. 2. There is considerable scatter around the relationship as presented in Behrenfeld et al. (1993*a*), and our own results indicate some disparities, particularly in lower than expected inhibition in some of the artificial light experiments. Overall, however, the agreement with the predicted inhibition was reasonably good, and none of our results would differ significantly from the predicted if the uncertainty in the predicted value itself were included. To a first approximation, then, the impact of UV-B on a variety of phytoplankton, including both marine communities and freshwater assemblages from Georgian Bay, would appear to have a simple linear dependence on the seasonal pattern of variation in total UV-B input, being maximal near the summer solstice in the north temperate zone.

It is important to note that our experiments, and those of Behrenfeld et al. (1993*a*), involved relatively long exposures of one-half to a whole natural UV-B photoperiod. Such experiments may only be representative of the relatively unusual situation in which mixing strength is minimal and the phytoplankton reside at the same optical depth for prolonged periods. There is a great need to pursue the kinetics of damage and recovery processes in natural phytoplankton (cf. Cullen and Lesser 1991; Lesser et al. 1994) so that UVR impact under more typical conditions of mixing and vertical motion can be assessed more realistically.

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Complex recruitment dynamics with Doppler-like effects caused by shifts and cycles in age-at-recruitment

K.N.I. Bell

Abstract: Even with constant production (N_0) and constant time-specific mortality (Z, daily), recruitment can vary greatly. A new variable-lag phenomenon modelled here generates temporal variation in recruitment from temporal shifts in age-at-recruitment (AAR; here synonymous with stage duration). The phenomenon is due to two components, one being analogous to the Doppler effect and the other an interaction of dAAR/dt with Z, and is distinct from those identified in previous contributions not focusing on temporal structure. Recruitment can be predicted from N_0, Z, and AAR, using either numerical simulation or, in many cases, the modified Doppler equation given here. Seasonal recruitment cycles predicted for a sinusoidal AAR function fitted to natural data, with constant larval production and constant Z (varied among, not within, model runs), were strongly peaked with a difference of 20% between maximum and minimum recruitments at $Z = 0$, to a difference of 2000% at $Z \approx 0.2$. The model gives indirect access to parameters difficult to measure in the field: if temporal variation is adequately described in two of the three input variables (N_0, Z, or AAR), differences (residuals) between predicted and observed recruitment can indicate an unaccounted pattern in the unknown.

Résumé : Même lorsque la production (N_0) et la mortalité spécifique du temps (Z, quotidienne) sont constantes, le recrutement peut varier considérablement. Un nouveau phénomène de décalage variable modélisé ici produit une variation temporelle du recrutement à partir de changements temporels touchant l'âge au recrutement (AAR; ici synonyme de durée du stade). Le phénomène est dû à deux composantes, une qui est analogue à l'effet Doppler, et est distincte des contributions antérieures qui ne sont pas centrées sur la structure temporelle. On peut prévoir le recrutement à partir de N_0, Z et AAR en utilisant soit une simulation numérique ou, dans nombre de cas, l'équation de Doppler modifiée présentée ici. Les cycles de recrutement saisonniers prévus pour une fonction AAR ajustée aux données naturelles, avec une production larvaire constante et un Z constant (qui variait d'un passage du modèle à l'autre, mais non dans un même passage), présentaient des pics prononcés, comportant des différences entre les recrutements maximum et minimum allant de 20 % à $Z = 0$, jusqu'à 2000 % à $Z \approx 0{,}2$. Le modèle donne indirectement accès à des paramètres difficiles à mesurer sur le terrain : si la variation temporelle est décrite adéquatement dans deux des trois variables d'entrée (N_0, Z ou AR), les différences (résidus) entre le recrutement prévu et le recrutement observé peuvent indiquer une caractéristique inexpliquée dans la variable inconnue.
[Traduit par la Rédaction]

Introduction

Although Roughgarden et al. (1988) comment that erratic fluctuations in abundance of exploited species have long standing as a topic of human interest, regular fluctuations, particularly those seen in species with seasonal variation in recruitment, are of equal interest. Explanations for recruitment variation over time are often sought in terms of variations in larval production (Robertson et al 1988; Robertson 1990), mortality (Houde 1987; Pepin 1989, 1991; Hoenig et al. 1990), or abiotic factors (Peterman and Bradford 1987). It is well known that increased stage duration (Houde 1987) or age-at-recruitment (AAR) can reduce recruitment, as is evident from the equation $N_t = N_0 e^{-Zt}$. It is also evident that, because t (in this context synonymous with AAR) is in the exponent, symmetrically increasing within-cohort variance of t increases recruitment because the increase in survival of that part of the cohort that experiences reduced AAR exceeds the reduction in survival of that part of the cohort that experiences increased AAR.

New to the present work is the link between a cycle or pattern of temporal change in AAR with a temporal pattern in recruitment. Profound changes in recruitment can result from temporal shifts in AAR, and acknowledging these may help to correctly identify other sources of recruitment variation.

Temporal change in AAR (i.e., a nonzero first derivative of AAR with respect to time, dAAR/dt) occurs naturally in *Sicydium* spp. (Bell et al. 1995), and has plausible origins in, for example, growth rate response to seasonal variations of temperature, food supply, wind, and mixing and may also respond to varying costs and benefits that an individual experiences by recruiting (Werner and Gilliam 1984). Growth rate and AAR are linked of course, but the dynamics that link them to recruitment success require exploration in terms of time, as AAR, "stage duration" as used by Houde (1987), or "postlarval duration" (Wellington and Victor 1992), etc. The terms and abbreviations important to this paper are summarized in Table 1.

Temporal changes in AAR will have unavoidable, and often

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Table 1. Abbreviations, definitions, and notation.

Abbreviation	Description
Variables	
AAR	Age at recruitment, usually the number of days from hatch to recruitment, but this term could be applied to the duration of any stage (synonymously with stage duration as used by Houde 1987). "One AAR" (etc.) may also be used to identify a unit of time approximately equal to age-at-recruitment
AAR curve	AAR as a function of hatch date unless otherwise qualified
DOY	Day of the year, numbered 0–365 (e.g., DOY = 0.5 means 12 noon on Jan. 1)
RDOY	Day of the year transformed to angular measure, usually radians (multiply DOY by $2\pi/365$)
LBBL	Barrels (≈80 L) of fish landed by the artisanal fishery for *Sicydium* spp. at Layou River in Dominica, used as an index of recruitment intensity
Subscripts	
hatch	Indicating that the main variable (e.g., DOY_{hatch}, $RDOY_{hatch}$) is taken at hatch (or estimated from regression against hatch date)
rcrt	Indicating that the main variable (e.g., DOY_{rcrt}, $RDOY_{rcrt}$) is taken at recruitment (or estimated from regression against recruitment date)
Labels and terms for models	
HDR model	Hatch date referenced model, i.e., the AAR function reflects expectation of AAR for given hatch date (this is the model used here in simulations)
RDR model	Recruit date referenced model, i.e., the AAR function reflects expectation of AAR for given recruitment date (no simulations done here with this model)
Absolute slopes	($\|dAAR/dt\|$); the absolute value of the slope of a curve. If, in an AAR curve, this exceeds 1.0 there will be bimodal age distributions in a HDR model or recruitment shadows in a RDR model
Cardinal points	The four cardinal points on the AAR function are extremes (minimum and maximum AAR) and nodes (the most positive and the most negative derivatives, $dAAR/dt$). These are useful for predicting phasing of the recruitment peaks and troughs under conditions of either high (using AAR) or low (using $dAAR/dt$) time-specific mortality
Surface	Recruitment surface, hatch surface; on model graphs, the locations where larvae hatch or recruit
Terms in periodic regression (Bliss 1958; Batschelet 1981; Bell et al. 1995)	
Mesor	The expected mean value of one full cycle of a periodic function. In estimation of the regression it functions like an intercept
Amplitude	The absolute distance from the mesor to the highest or lowest points in a periodic function
Others	
t	Time (in days)
S	Survival, time-specific (in units·day^{-1}); $= (N_t/N_0)^{1/t}$
M	Mortality, time-specific (in units·day^{-1}); $= 1 - (N_t/N_0)^{1/t}$
S_{cum}	Survival, cumulative; $= S^t$
M_{cum}	Mortality, cumulative; $= 1 - S^t$ (note that M^t is nonsensical)
N_0	Numbers entering a stage (e.g., numbers hatching)
N_t	Numbers completing a stage (e.g., numbers recruiting)
Z	Instantaneous total mortality ($= -\ln S$ (Ricker 1975), in units·day^{-1}). Outside the fisheries context the expression e^{-Z} could equate with e^r
Cohort	A group of individuals having in common some identified feature of interest, such as the same hatch or recruit date, according to context
For Doppler equations	
F_s, F_p	Frequency of a signal as emitted by source (F_s), and as perceived (F_p) by observer; for light or sound this is Hertz or cycles per unit time; for recruitment this is individuals per unit time
POSITION	Distance (or time) from source to observer
C	Rate of propagation of a signal between the source and observer

Note: Many names and labels use acronymic combinations of letters; therefore, multiplication is explicitly indicated by a times sign or use of brackets where it is otherwise ambiguous.

large, consequences for recruitment dynamics. These consequences arise from both *i* a variable-lag effect and *ii* its interaction with time-specific mortality. The variable-lag effect seems to be the first demonstration of a population-dynamic example of the Doppler effect. The combined action of *i* and *ii* can be modelled either by numerical simulation (which requires very few assumptions) or by the modified Doppler equation. Although the explorations below assume constancy over time of both reproductive effort and time-specific mortality rates, any variation in these would contribute to the final pattern; a potentially useful implication is that this affords an approach to investigating them indirectly. The existence of

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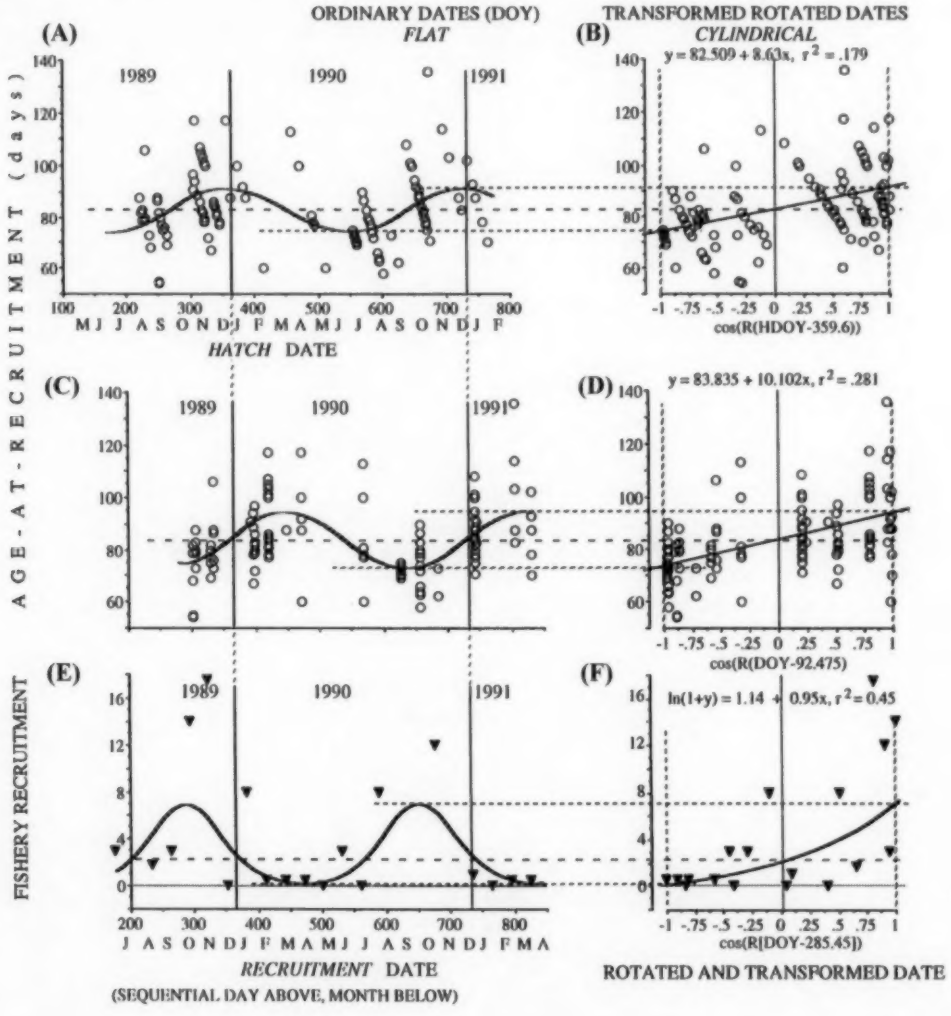
Fig. 1. Seasonal variations in AAR (as function of hatch date and recruitment date) and recruitment for *S. punctatum* in Dominica, W.I. Panels A, C, and E are positioned so that the same dates are vertically aligned. Dates are indicated as day numbers beginning with January 1, 1989. Curves are least-squares periodic regressions given in Table 2 (all significant). (A) AAR vs. hatch date. Inclination of groups of points is because samples were collected at recruitment, not hatch, and $x_i + y_i$ = hatch date + AAR = collection date. (C) AAR vs. recruitment date. (E) Recruitment, in units of barrels (LBBL, ≈80 L) as indicated by the fishery at Layou river. Because periodic regression is uncommon I have presented two types of plots for clarity: "flat" (A, C, and E) and "cylindrical" (B, D, and F); the *x* axes for the cylindrical plots are DOY, first corrected by the lag (fitted rotation, worth 1 df), then converted to a standard (radians or degrees) angular scale, and finally converted to a cosine. Broken horizontal lines relate the mesor and amplitude of periodic regressions to normal regression on transformed variables.



seasonal peaks in recruitment and their link with a seasonal variation in AAR have implications for the evolution of seasonal bias in reproduction.

In their discussion of recruitment, Roughgarden et al. (1988) recognised not only the complexities of two-phase (benthic–pelagic) life cycles, but also, implicitly, the value of using a natural change in niche and habitat (settlement) as a recruitment definition. Settlement is the outcome of a biological decision (Werner and Gilliam 1984) that may be thought to integrate many factors impinging on recruitment and fitness. Recruitment for *Sicydium punctatum* refers to the migration of postlarvae from the sea into freshwater, and this migration coincides with settlement and also a directed fishery. Seasonally, numbers of recruiting *S. punctatum* in Dominica, West Indies, vary from near nil to spectacular surplus. The temporal variation in AAR and recruitment provided the example for this study, and the temporal range in AAR was the basis for the input to simulations.

Although Bell et al. (1995) showed the first example of seasonally structured variation directly measured in AAR (Fig. 1, Table 2), it was not unexpected because seasonal growth rate variations had previously been shown and even

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Table 2. Regression equations describing seasonal variations in AAR (age-at-recruitment) with respect to (A) hatch dates, (B) recruitment dates, and (C) fishery yield (reported as barrels, LBBL, ≈80 L) as an index of recruitment, with respect to recruitment dates (illustrated in Fig. 1).

(A) *Sicydium punctatum* AAR by hatch date			
$AAR_{hatch} = Int + \beta_1 sin(RDOY_{hatch}) + \beta_2 cos(RDOY_{hatch})$	r^2	n	p
(*i*) $AAR_{hatch} = 82.5 - 0.794 sinRDOY_h + 8.593 cosRDOY_h$ $p = 0.7462$ $p = 0.0001$ peak at DOY 359.6, amplitude = 8.63	0.179	117	0.0001
which can be rewritten as			
(*ii*) $AAR_{hatch} = 82.5 + 8.63\cos(2\pi(DOY_{hatch} - 359.6)/365)$			
which closely approximates the (more conservative) function applied in simulation:			
(*iii*) $AAR_{hatch} = 81.3 + 6.4\cos(2\pi(DOY_{hatch} - 0)/365)$ peak at DOY 365 or DOY 0, amplitude = 6.4			
(B) *Sicydium punctatum* AAR by recruitment date			
$AAR_{rcrt} = Int + \beta_1 sin(RDOY_{rcrt}) + \beta_2 cos(RDOY_{rcrt})$	r^2	n	p
$AAR_{rcrt} = 83.835 + 10.1 sinRDOY_{rcrt} - 0.21 cosRDOY_{rcrt}$ $p = 0.0001$ $p = 0.9243$ peak April 2, amplitude 10.102	0.28	117	0.0001
(C) *S. punctatum* recruitment (LBBL) by recruitment date			
$\ln(1+LBBL) = Int + \beta_1 sin(RDOY_{rcrt}) + \beta_2 cos(RDOY_{rcrt})$	r^2	n	p
$\ln(1+LBBL) = 1.13 - 0.93 sinRDOY_{rcrt} + 0.19 cosRDOY_{rcrt}$ $p = 0.0024$ $p = 0.4541$ peak at day 285.4 (Oct. 12)	0.45	19	0.0079

Note: See Table 1 for abbreviations. These data were collected in Dominica, W.I., 1989–1991. Regression A (*iii*) was used as simulation input; the expressions "– 359.6" and "– 0" are nearly the same thing for a 365-unit cycle. A minor (<5%) component of the goby-fry fishery consists of species other than *S. punctatum*.

incorporated into estimation of the von Bertalanffy growth function (Pitcher and MacDonald 1973; Pauly 1985), and the estimation of Z from catch curves (Pauly 1990). Also, seasonality exists in environmental variables, and there is within-cohort, individual variation in AAR (Victor 1982, 1984, 1986*a*, 1986*b*; Chambers and Leggett 1987; Wellington and Victor 1989, 1992; Cowen 1991; McCormick 1994); these also support the possibility of variation in AAR over time.Therefore, it is likely that many taxa (of fish particularly, but also of insects and plants) may show some seasonally structured variation in AAR.

Temporal change in AAR affects recruitment in unexpected ways, so the purpose here is to develop a framework to understand and predict these. If factors such as reproduction or survival vary over time they will also contribute to recruitment variation, but they are held constant in explorations below to isolate the effects of changes in AAR.

In this paper, I show how temporal AAR variation can influence recruitment. The arguments are mathematical rather than biological and, therefore, are not restricted to fish. I model the mechanisms graphically, showing qualitative differences that depend on levels of variation in AAR and also on the life-history stage where the AAR is assumed to be set or ordained. I show graphically the similarity of one model (hatch-date referenced (HDR), which assumes that AAR is set at hatch) to the Doppler effect. I use numerical simulations to explore the dynamics of this model using various functions, including AAR data for *S. punctatum*, as input to the model. I show that the phenomenon by which temporal pattern in AAR generates temporal pattern in recruitment can also be modelled by modified Doppler equations. Sinusoidal variation is the simplest structure for cycles (cf. Pitcher and MacDonald 1973; Pauly 1985) and, therefore, a good starting point for describing temporal changes in AAR, although the prediction methods are not constrained to sinusoidal variations. The dynamics apply to numbers entering and exiting any stage of variable duration, so where I use "hatch" and "recruitment" it can be read as "entering" and "exiting" a stage.

Theory and models

The theoretical basis for a relationship of changing AAR to changing recruitment is twofold, arising out of (*i*) the derivative of AAR with respect to time, or $dAAR/dt$, and (*ii*) the value of AAR. (*i*) The temporal spacing of hatch cohorts is disrupted (see Figs. 2–4) by changing AAR: temporal spacing at recruitment (apparent frequency) differs from original spacing at hatch (source frequency). This is strictly due to $dAAR/dt$ and is analogous to the Doppler effect, which is the alteration of perceived signal frequencies due to displacement of either the source or observer during either the production or reception of the signal. Doppler algebra is given in the section on HDR models. (*ii*) A constant time-specific mortality rate (Z) compounded over varying periods of time gives exponentially varying cumulative or total mortality, so that temporal changes in AAR can cause much greater temporal changes in recruitment. This arises from the familiar equation:

$$(1) \quad N_t = N_0 e^{-Z(t-0)}$$

where N represents cohort size, $t - 0$ is the number of time units (equivalent to AAR) that separate N_0 and N_t, and Z is the natural

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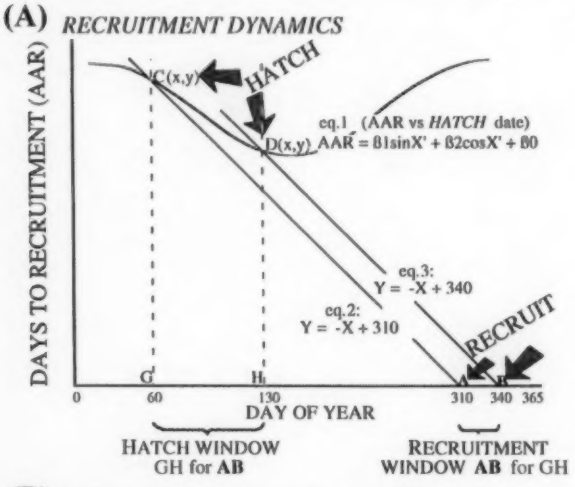
Fig. 2. (A) Recruitment dynamics, HDR model (hatch-date referenced), moderate slopes only (all $|dAAR/dt| \leq 1.0$; see text). This is the situation used in simulations. The x axis represents one annual cycle. Recruitment over the period AB (arbitrarily chosen) results from larval production over period GH. By definition, the number of days to recruitment declines by 1 each day of an individual's life, so slopes of figure eqs. 2 and 3 are –1. Rate of recruitment (recruits arriving per unit time) is more intense over the period AB than is hatching (per unit time) over the corresponding period (GH), but the opposite occurs in other seasons. Note that vertically moving the AAR curve changes the interval between hatch and recruit times, but the extent of convergence or divergence of recruitment is affected only by the slope of the curve. (B) Doppler analogy: a stellar example fitting the HDR model with a sinusoidal function would be a binary star system: light from the bright star would be red-shifted (longer wavelength, lower frequency) when receding and blue-shifted when approaching. The distortion of a source frequency by relative motion of the source and observer is analogous to the distortion of rate of larval production by temporal shift in the lag from hatch to recruitment (or change in AAR). The speed of light is indicated by a 45° line (units distance/time or light-year/year); similarly, the speed of transmission of a larval pulse is –1 (units day remaining/day elapsed).



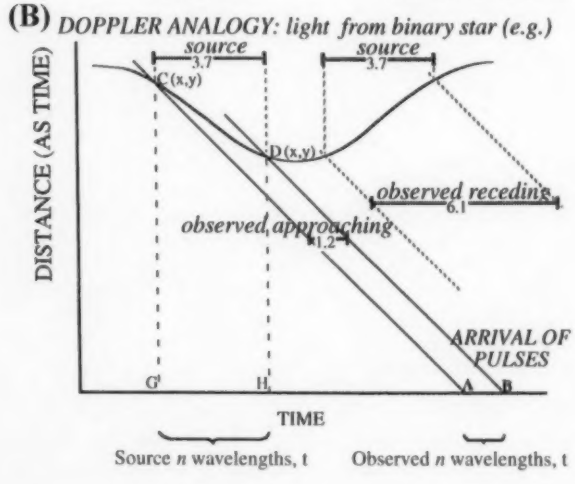
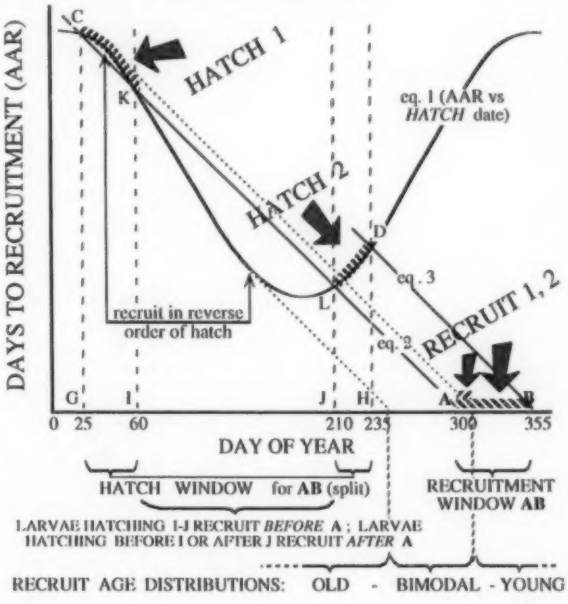


Fig. 3. Recruitment dynamics, HDR model (hatch-date referenced), with high slopes (extremes of $|dAAR/dt| > 1.0$; see text and previous figure legend). Where $dAAR/dt < -1$, recruits show bimodal age distribution at some times. The mechanics are similar to the previous example, except that because of the high negative slope (i.e., $dAAR/dt < -1$) it is possible to have multiple intersections of the AAR curve (figure eq. 1) with larval trajectories (figure eqs. 2, 3, etc.), and this causes the hatch window corresponding to the recruitment window AB to be split (GI and JH), giving rise to bimodal age distribution in recruits near A. Larvae hatching in the interval IJ (within GH) recruit prior to time A (hatch order disrupted), and recruitment is in precisely the reverse order of hatches when $dAAR/dt < -1$.



logarithm of survival ($S = e^{-Z}$, $Z = -\ln S$) with the sign changed (Ricker 1975). Outside the fisheries context the expression e^{-Z} could equate with e^{r}. N_0 and N_t are the beginning and end of a period represented by AAR or stage duration (and may be used for numbers at an egg, larval, juvenile or any other stage, provided that N_0 precedes N_t).

To conceptualize these dynamics, assume that AAR is substantially determined at the date of occurrence of some major event in the early life history. Although any dates (e.g., hatch, recruitment) in the hatch to recruitment period may have some influence on AAR, the beginning (hatch date) allows the most tractable models, and there is at present little basis on which to prefer other models. Therefore, this paper uses HDR models for simulations, but for contrast I shall also describe recruitment date referenced (RDR) models. The results of these two are qualitatively quite different. HDR models are more plausible than RDR models because the hatch date (predictor) precedes the recruitment date (predicted), instead of the other way around. To illustrate the main qualitative differences in the range of possibilities, three basic graphic models are presented: HDR models with AAR curves having maximum absolute slopes ($|dAAR/dt|$) less than 1.0 (Fig. 2) or exceeding

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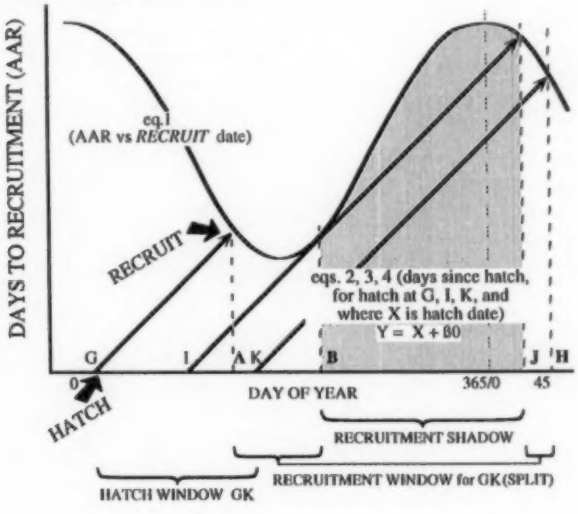
Fig. 4. Recruitment dynamics, RDR model (recruitment-date referenced), with extremes of |dAAR/d*t*| > 1.0. (RDR models are not applied in the simulations.) If AAR is thought of as being determined at recruitment date instead of hatch date, qualitatively different dynamics are generated if maximum slope of the AAR curve also exceeds 1. Larvae hatched in interval GI gain age at one day per day, until intersection with the AAR curve, which now acts as a recruitment surface; they recruit in interval AB. However, recruitment is zero under segments of the AAR curve from above B, where maximum slope first exceeds 1, to the point where the next larval trajectory (line having slope = 1) passing the AAR curve above B intersects with the AAR curve (above J). Thus, while production during GI is recruited over the similar interval AB, the point marked I is the hatch date where, suddenly, time to intersection with the AAR curve becomes very long, so there is no recruitment between B and J (shaded). Larvae hatched between I and K recruit between J and H, a narrower interval, giving (assuming $S = 1.0$) a rise in recruitment rate not due to any change in larval (stage$_0$) production rate.



1.0 (Fig. 3) and an RDR model with AAR curve having maximum absolute slope exceeding 1.0 (Fig. 4).

The main components of the graphic models are hatch surfaces and recruitment surfaces. Larvae originate on the hatch surfaces and, with accumulation of age, progress on a 1:1 line (negative in the HDR model, positive in the RDR model) toward the recruitment surface. The first intersection of a larval trajectory with a recruitment surface indicates by definition a recruitment event, but any intersection of a larval trajectory after hatch with the hatch surface has no consequence (recruitment surfaces are defined as opaque, hatch surfaces transparent).

Hatch date referenced AAR (HDR model)

In the HDR model, a hatch event is represented (Fig. 2) on the AAR curve by x_i (date) and y_i (the ordained AAR for that hatch date, AAR_{hatch}). All (x_i, y_i) on the graph denote (date, (AAR – age)); the AAR curve is consistent with this because a hatching larva has age = 0. The function for AAR_{hatch} can be readily estimated from data by periodic regression (Bliss 1958; Batschelet 1981; Bell et al 1995) of AAR against hatch date. A line with slope –1 originating on the AAR curve represents the (date, (AAR – age)) trajectory of a larva, and it will intersect with the x axis ($y = 0$) at the recruitment date. These lines can be used to graphically locate the hatching interval corresponding with any recruitment interval, or vice versa. Figure 2A shows the simplest situation: for an AAR_{hatch} curve with $-1 <$ slopes < 1 (this is the model used with *S. punctatum* data for simulations below). The spacing of larvae at recruitment can differ markedly from that at hatch (Fig. 2).

The Doppler effect analogy (for the HDR model) The model describing recruitment variation resulting from varying AAR (Fig. 2A) is analogous to the Doppler effect (Doppler 1842), named for its discoverer. The Doppler principle explains how a frequency shift (e.g., in light or sound) is generated by relative motion of source and observer in space (Fig. 2B). The principle is important in astronomy for estimating stellar motion from shifts in absorption lines. The principle can be expressed as

$$(2) \quad F_p = F_s(1 - (\mathrm{dPOSITION}/\mathrm{d}t)/C)$$

where F_s, the frequency generated at source, is perceived as F_p at some distant point according to the relative radial motion dPOSITION/dt (negative if source approaching, positive if receding) compared with C, the speed of signal propagation (Otten 1992). POSITION is the distance (or the time required for the signal to traverse that distance at velocity C) between the signal source and the observation point where it is perceived. The variate POSITION is most conveniently expressed in units of time separating the source and observer given the velocity C (for example, light-years); the time coordinate for the arriving signal is simply $t_p = t_s$ + POSITION. I'll ignore the correction for relativity. A similar effect could occur if the speed of transmission changes (for sound, this would occur if air density changed) or if the signal takes a longer route.

A changing AAR is analogous to changing position of a signal emitter relative to an observer, because it varies the time until recruitment. Like a Doppler effect, the perceived (recruitment) frequency differs from the hatching frequency only when AAR (or POSITION) is changing; when dAAR/dt = 0 the frequency of recruitment reflects the frequency of hatching.

The Doppler equation can be used to calculate recruitment by treating as frequencies the rates of larval production (F_s) and subsequent recruitment (F_p), and taking C to be unity (absolute value of d(age)/dt, or the slope of signal trajectory in Fig. 2). To incorporate mortality, the Doppler formula as used for light or sound requires extension by multiplying F_p (as obtained above) by time-specific survival exponentiated to the time over which the fish (etc.) is exposed to such mortality:

$$(3) \quad \mathrm{Recruitment} = F_s(1 - (\mathrm{dPOSITION}/\mathrm{d}t)/C)S^{AAR}$$

and of course the date of recruitment is obtained by adding AAR to the hatch date. Where mortality is not constant over time, the cumulative product of the daily survivals for the hatch to recruitment period ($S_0 \times S_1 \times \ldots \times S_{\mathrm{recruitdate}-1}$) should be used to replace the expression S^{AAR} (eq. 3).

The recruitment equation based on the Doppler equation gives virtually the same results as a numerical simulation model used below (the simulation results are slightly more

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noisy because of recruitment-date overlaps and misses). However, results from the numerical simulations are relied on, because simulation does not assume a Doppler analogy. The simulation data illustrate the phenomenon and provide a basis to evaluate the predictions from the modified Doppler equation (eq. 3) and the analogy. It will be shown that the modified Doppler equation allows easier prediction of recruitment from temporal variations in AAR, provided that $|dAAR/dt|$ is less than about 0.5.

Hatch date referenced AAR with rapid change (HDR + steep slope)

If the most negative slope of the AAR_{hatch} curve is lower than −1, some recruitment periods will correspond to two non-adjacent hatch intervals (Fig. 3). The consequence is a bimodal or discontinuous age distribution in the recruits in some parts of the cycle (although where recruit ages are potentially bimodal, mortality would affect the older cohort for a longer time and exponentially reduce its proportion). For a portion of the AAR curve with slope less than −1, recruitment order will be the reverse of hatching (Fig. 3). A special case would exist with any kind of AAR curve with slopes for some segment exactly equal to −1.0 for some time; convergence would be infinite, so that all larvae hatching (or individuals entering the stage of interest) on this segment would recruit (or complete the stage) at a single instant. In more complex functions, high $dAAR/dt$ may be more commonly seen than in sinusoidal functions with the same overall range, and if so these recruitment dynamics may prevail during parts of the cycle.

Recruitment date referenced AAR with rapid change (RDR + steep slope)

A brief caveat is that AAR could be treated as a function of dates other than hatch date, and this could result in qualitative differences where AAR curves have slopes steeper than ±1. For example, I could view AAR as being determined at recruitment date instead of hatch date. The AAR function, AAR_{rcrt}, needed as a model input would then have been estimated from data by regressing AAR against recruitment date. In the RDR model's diagram (Fig. 4) the coordinates x and y denote date and age, respectively, and the vertical lines would then relate to the recruitment event. Positive 1:1 lines would lead from hatch at $(x, 0)$ until recruitment, indicated by the intersection of these with the AAR_{rcrt} curve. The value of Y on this 1:1 line is (by definition in this model) equal to the larva's age at time X (whereas, in the HDR models, Y on a −1:1 line represented time remaining, at time X, until recruitment). Larvae cannot pass through the recruit surface, which here is the AAR_{rcrt} curve, but must recruit at first encounter. Because of this, where the AAR_{rcrt} curve has a slope >1, seasonal recruitment shadows can be formed and larvae recruiting after the shadow are then relatively old. In contrast, a HDR model could give bimodal age distributions in some seasons because of slopes less than −1. In the HDR model the recruitment surface was the x axis (which is flat and cannot project shadows on itself, so its opacity is of no consequence here) and the AAR_{hatch} curve was "transparent" so that hatched larvae are uninfluenced by re-encountering the curve after their own hatch dates; but in the RDR model the (opaque) recruitment surface is the AAR_{rcrt} curve, which can project shadows on itself because of its shape, and the hatch surface is flat and cannot be re-encountered.

Other models

Other geometric models could describe systems where AAR is determined at points partway through the hatch-to-recruitment period or other stage of interest. This matters if the early life-history points that best explain temporal pattern in AAR turn out to be intermediate between hatch and recruitment. A brief discussion is warranted because future work may be able to confirm this and identify the timing of the strongest influences on AAR.

For example, the "halfway" model: if the determination of AAR was deemed to occur halfway through the stage, the x axis would be both the hatch and the recruitment surface; the AAR function would be a half-AAR curve determined by regression of Y_i (= $0.5(AAR_i)$) against X_i (= recruit date − $0.5AAR_i$). A larval trajectory would begin on the x-axis (as in RDR model) and trace a positive 1:1 line until contact with the half-AAR function, would reflect off this and trace a negative (−1:1) line until intersecting with the x-axis (recruitment, as in the HDR model). Such a model would operate by predicting the AAR associated with the point of reflection. Because it has both negative and positive 1:1 lines for larval trajectories, it could produce both bimodal age distributions and recruitment shadows (in other words could produce the qualitative effects of both HDR and RDR models), but the half-AAR curve in the model would require real $dAAR/dt$ of over 2.0 to yield the rate of change over 1.0 on the half-AAR curve that would be needed to produce these extreme effects.

Models referenced to intermediate points are more cumbersome to simulate, and at present not significantly more plausible, than the HDR models which occupy the bulk of this paper.

Methods

Source of data

The source of AAR data for simulation (numerical) and calculation (using modified Doppler equation) input is natural (Bell 1994; Bell et al. 1995), so the simulations are based on realistic variation. While it is likely that AAR might cycle in many species, it has been clearly demonstrated only in *S. punctatum* and *S. antillarum*. Sinusoidal variation in AAR, as seen in *Sicydium* spp. in Dominica, is one among many possible patterns of dynamically varying AAR.

Sicydium spp. are diadromous gobies, spawning in rivers and spending a postlarval period in the sea (see Fig. 2 and description of life cycle in Bell et al. 1995) before a migration back to and settlement in rivers (recruitment). Reproduction occurs year round, but recruitment is episodic and synchronized near the last lunar quarter (Bell et al. 1995). The highest recruitment (and fishery yield) occurs in the fall and the lowest in the spring. For *Sicydium* spp., recruitment coincides with settlement and is also the stage at which a fishery occurs, which was convenient because it provided quantitative estimates of recruitment. Thus, AAR for *Sicydium* spp. is like age-at-settlement or at metamorphosis, as used by other authors (Victor 1986*b*; Chambers and Leggett 1987; Radtke et al. 1988; Robertson et al. 1988; Wellington and Victor 1989; Cowen 1991; Markle et al. 1992; Robertson 1992; McCormick 1994). Biologically based definitions, because they recognise choices made by individuals, can suggest elements of the

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decision process or constraints involved in recruitment (e.g., Bell et al. 1995, Fig. 7).

The periodic regression equation (Fig. 1, Table 2) presented for *S. punctatum* in Bell et al. (1995) is very close to the more conservative (lower amplitude) cyclic AAR function used here, which is

$$(4) \quad AAR = 81.3 + 6.4\cos(RDOY_{hatch} + 0),$$

where DOY is day of year, hatch denotes hatch date, RDOY is the DOY transformed to a cyclic variable in units of radians (RDOY = DOY × 2π/365), and adding 0 indicates a nil phase lag with the calendar year (year as a circular variable). Periodic regression (Fig. 1, Table 2) is formally identical to linear regression of Y against a cosine transform of the X variable after adding a fitted lag (Bliss 1958; Batschelet 1981; Bell et al. 1995). The periodic regression of AAR against time of year is highly significant ($p = 0.0001$, $n = 191$). Although there is considerable variation among individuals at each date, the r^2s from equations based on individual data are conservative in the sense that they are much stronger predictors of the mean value for each sample date.

Modelling for the present purposes is best done using time or age, as, for example, stage duration (Houde 1987) or AAR. Conversions from size to age can sometimes give very misleading indications of relative ages because (*i*) there is an individual component to growth rates, and (*ii*) as seen in the *Sicydium* spp. data (Fig. 7 of Bell et al. 1995), the slope in an among-cohort regression of (using cohort means of) size-at-recruitment against AAR can be negative even though the size versus AAR slopes in within-cohort (using individual values) regressions are positive. Therefore, time should be estimated using a method (e.g., otoliths) that is independent of size.

Simulation using HDR models

Given an AAR-at-hatch curve (as in Fig. 2), the graphic methods make it possible to identify the production interval(s) corresponding to any chosen recruitment interval. Projecting temporal trends in recruitment using this method would be possible but tedious. Therefore, I further explore the HDR model using a numerical simulation program that incorporates the compounding effects of mortality over time on larvae hatching each modelled day. Although I show below that the modified Doppler equation can do the same thing in most circumstances, the numerical simulation method requires no assumptions other than the inputs to the simulation; this allows the results of numerical simulation to also serve as a test for the modified Doppler equation.

The simulations show the recruitment variation that can result from a situation where the following prevail: (*i*) AAR varies as a function of hatch date; (*ii*) larval output is held constant over time; and (*iii*) time-specific survival rates are held constant over time.

The simulation structure (Fig. 5) is based on each larva having an expected recruitment date, and the total number of recruits on a particular day being the sum of all larvae having that expected recruitment date, less mortalities. Cohort size at recruitment is given by eq. 1 or similar language:

$$(5) \quad N_t = N_0 e^{\ln(S)AAR}.$$

Of course, recruitment date is equal to hatch date plus AAR.

For most simulations (Fig. 5), I used an AAR function (eq. 4) with parameters close to those obtained from otolith data (Table 2) for *S. punctatum* (Bell et al. 1995). Scaled versions of this function, and a step function, were also used. For each day in the simulation, ages-at-recruitment, date of recruitment, and cumulative survival were calculated for a hatching cohort of 10^6 pseudoindividuals. Recruits were accumulated for each recruit date (day of year, treated as a cycle of period 365 and thence disregarding the year of recruitment) while the program cycled through an entire year of hatch dates. Fractional individuals were permitted. The simulation program wrote results to a file. Integer effects of date caused zeroes or approximate doubling when changing AARs passed whole numbers, so smoothing of plots was done by taking 28-day running means. The 28-day span was reasonable because recruitment of *S. punctatum* is episodic and lunar phased.

Results (simulations using the HDR model)

Simple change in age-at-recruitment

The simplest variation function used for AAR in a HDR model is a step function (Fig. 6). This is probably not natural, but it is instructive. The simplest case of this simplest function is where time-specific (daily) survival = 1.0 (Fig. 6, upper output panel): all cohorts have the same cumulative survival to recruitment, because $S^n = 1.0^n = 1.0$. Abrupt steplike increases in AAR are followed, after a delay approximating AAR (because that is how long it takes a signal to travel from hatch to recruitment), by abrupt decreases in recruitment (briefly to zero, because all recruitment has been postponed by the increase in AAR). The decrease is of a duration approximately equal to the change in AAR, so that a positive difference of 10 days in AAR results in a decrease in recruitment that lasts about 10 days. Restoration of previous AAR is just a change in the other direction: a 10-day decrease in AAR causes a (delayed) increase (briefly to twice the original level here because each recruit date is now collecting from two hatch dates) in recruitment, which lasts about 10 days (because that is for how long each recruit date collects from two hatch dates).

If we also add (Fig. 6, bottom panel) mortality ($S < 1.0$) compounded over AAR, cumulative survival will vary negatively and exponentially with AAR (eq. 5). The effects are similar to the zero-mortality (previous) situation, but recruitment is also diminished for the duration of the increase in AAR.

Thus, an abrupt change of AAR from one value to another affects recruitment (Fig. 6, bottom panel) in two ways: (*i*) transient spiky effects because the spacing of recruits is changed from their spacing at hatch and (*ii*) sustained effects (lower plateau in bottom panel) due to the compounding of mortality over the (changed) time represented by AAR. These two effects are both important but are less distinguishable when changes in AAR are gradual.

Sinusoidal change in age-at-recruitment

The sinusoidal AAR function used for the HDR simulations here approximates that found for AAR of *S. punctatum* (Fig. 1, Table 2). Day-to-day fluctuations in S or N_0 could be easily incorporated, but they play no role whatever in the simulations presented here.

Model runs over a range of daily survivals produce a range

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Fig. 5. Flowchart for numerical simulation of effect of AAR variation on recruitment (numbers), showing steps taken by the program.

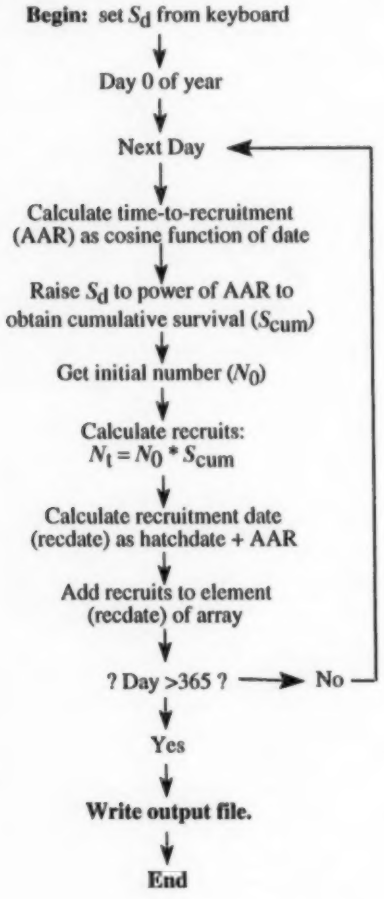
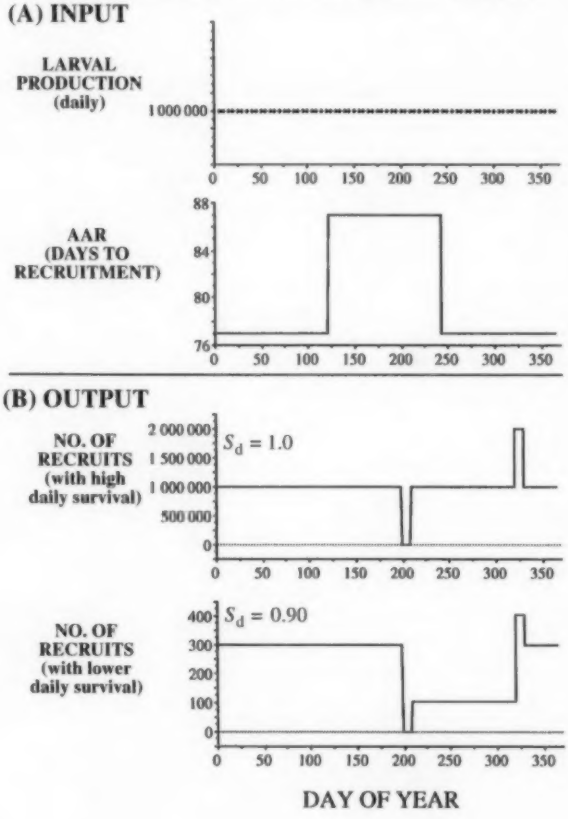

Begin: set S_d from keyboard
Day 0 of year
Next Day
Calculate time-to-recruitment (AAR) as cosine function of date
Raise S_d to power of AAR to obtain cumulative survival (S_{cum})
Get initial number (N_0)
Calculate recruits: $N_t = N_0 * S_{cum}$
Calculate recruitment date (recdate) as hatchdate + AAR
Add recruits to element (recdate) of array
? Day >365 ?
No
Yes
Write output file.
End

Fig. 6. Effects of abrupt changes in AAR (arbitrary, unnatural data). Reproduction is constant (10^6·day^{-1}) for this simulation. Upper graphs (A) show inputs to model: larval production and AAR over time. Lower graphs (B) show simulated recruitment patterns obtained with daily survival rates as noted on each graph.



of recruitment profiles (Fig. 7). At $S = 1.0$ the variation (range/minimum) is 30%. The recruitment curve is a symmetrical hill with a maximum nearly coincident with the quickest AAR, but as will be seen this is a special case (mean AAR ≈ 0.25 × 365 days) and will not turn out to be a generality. At $S = 0.97$ the variation exceeds 50%, and the curve is losing its left–right symmetry as a trough of low recruitment begins to show on the left side. As S is reduced, the trough becomes a flat minimum that becomes lower and wider; the peak becomes relatively higher, narrower, and shifted to the right. At $S = 0.7$ the variation is 9600%, because recruitment is virtually zero during the winter and spring, and forms a narrow, relatively tall peak in the fall. (In field observations, minimum values may be low and erroneously interpreted as zeroes, which would inflate the apparent geometric variation, but the output of simulations is not subject to the same observational error.) Initially ($S = 1.0$) the peak is near day 180, the trough near day 0 (or 365). Progression of both of these is rapidly to the right as S decreases even minutely from 1.0, but the progression slows near $S = 0.90$, with little change down to $S = 0.60$.

Recruitment maxima and minima in scaled AAR curves

Scaling of the AAR function (Fig. 8) also affects the recruitment profile. I ran simulations using the naturally derived AAR function (eq. 4) multiplied by 2, 1, 0.5, and 0.25. This preserved the same variation relative to the mean. Because we saw in Fig. 7 that temporal locations of peaks and troughs changed little below $S = 0.90$, I plot only $S = 1.0$ and $S = 0.70$ to show recruitment patterns resulting under conditions of zero and substantial time-specific mortality.

The results show that, even though scaling preserves the seasonal phase of the AAR functions themselves, the seasonal phase of maxima or minima of recruitment are profoundly shifted by scaling. With doubled AARs (150–175 days to recruitment), curves are shifted to the right for both $S = 1.0$ and $S = 0.7$. With halved values the peak at $S = 1.0$ precedes the AAR minimum, while the $S = 0.70$ peak follows it by about 40 days. With quartered AARs, the $S = 1.0$ peak is further still to the left, and the $S = 0.70$ peak is only slightly later than the AAR minimum. As AAR is scaled downward, the recruitment peaks move to the left (occur earlier), and the tendency for mortality to distort the sinusoidal zero-mortality recruitment profile to a log-sinusoidal form is reduced.

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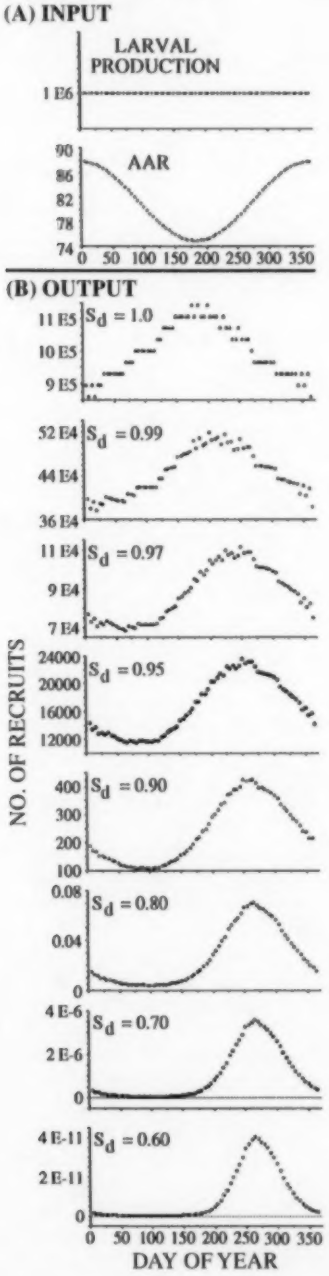
Fig. 7. Simulation of effect of seasonally shifting AAR on recruitment. (A) Inputs to model are AAR (days to recruitment, natural data) and larval production (10^6·day^{-1}; constant), and time-specific survival input (constant over time), is noted on each output graph. (B) Output graphs show simulated recruitment profiles (28-day moving averages contribute a constant additional recruitment lag of ~14 days) obtained with each S. At daily $S = 1.0$, all variation is due to Doppler-like effects giving a recruitment peak near day 180, but the increasing role of mortality shifts the recruitment peak to the right, stabilizing where mortality effects dominate (in this example near day 265).



Discussion

Predicting timing or recruitment peaks from AAR curves

If we expected timing of peaks or valleys to always occur at the AAR minimum and maximum, the results will have seemed counterintuitive. At $S = 1.0$ ($Z = 0$) there is no difference in S_{cum} with varying AAR ($1^n = 1$). Here, the timing of recruitment peaks is purely determined by a Doppler-like effect, which causes convergence of multiple-day hatch cohorts on fewer recruitment days (Fig. 2) when AAR is decreasing. When AAR is increasing, divergence occurs instead. However, when time-specific mortality rates exceed 0 ($S < 1.0$), they interact with shifts in AAR to draw recruitment peaks away from their zero-mortality positions toward the recruitment dates corresponding to the lowest AAR, i.e., toward the date represented by the expression "hatch date + AAR" where hatch date is at the minimum AAR. With $S < 1.0$, fewer recruits result (eqs. 1, 5) from a slow-recruiting cohort (high AAR or t) than from a faster recruiting (shorter AAR) cohort; if recruitment occurs at a constant size then this would also be true for slower or faster growing cohorts. That this happens is nothing to do with size- or age-dependent mortality but is simply the result of accumulation of time-specific mortality, which is exponentially affected by AAR.

With diminishing S (increasing M and Z) the Doppler-like effect is increasingly swamped by variable survival. The seasonal recruitment profile thus shifts, as mortality increases from zero, from being Doppler dominated to being mortality dominated. This suggests how recruitment peak timing might be determined from the AAR_{hatch} curve.

On an AAR curve four "cardinal" points (Fig. 9) are useful for quick visual prediction of recruitment profile phasing. These are the extremes (maximum and minimum AAR) and the nodes (the most negative and the most positive derivatives, dAAR/dt). The AAR extremes are useful in prediction when mortality is high (e.g., $S \leq 0.7$), and the derivatives are useful when mortality is near zero ($S \approx 1.0$). On a sinusoidal curve, the cardinal points are equally spaced horizontally, but their projections (along larval trajectories or lines of slope = –1) onto the x axis fall on points which are less equally spaced with increasing amplitude of the AAR curve.

To predict recruitment phasing from the AAR curve, lines are drawn with slope –1 from the cardinal points (Table 1, Fig. 9). The intersections with the x axis (i.e., at $Y = 0$) mark the times of maximum and minimum recruitment, for either low mortality (lines from extremes of dAAR/dt) or high mortality (lines from extremes of AAR). At very low mortality rates ($S \approx 1.0$) the recruitment peak will be approximately one AAR after the most negative derivative, and the recruitment trough will be approximately one AAR after the most positive derivative. At higher mortalities (e.g., $S \approx 0.7$) the recruitment peak will approach a point approximately one AAR after the AAR minimum value, and the recruitment trough will approach a point approximately one AAR after the AAR maximum value. The transition from $S \approx 1.0$ to 0.7 (Fig. 7) shows joint influence, and cannot be predicted using simple geometry. In other kinds of curves or complex AAR cycles with multiple peaks and troughs, the same rules for prediction would apply.

Where the recruitment minima are wide troughs, the graphical

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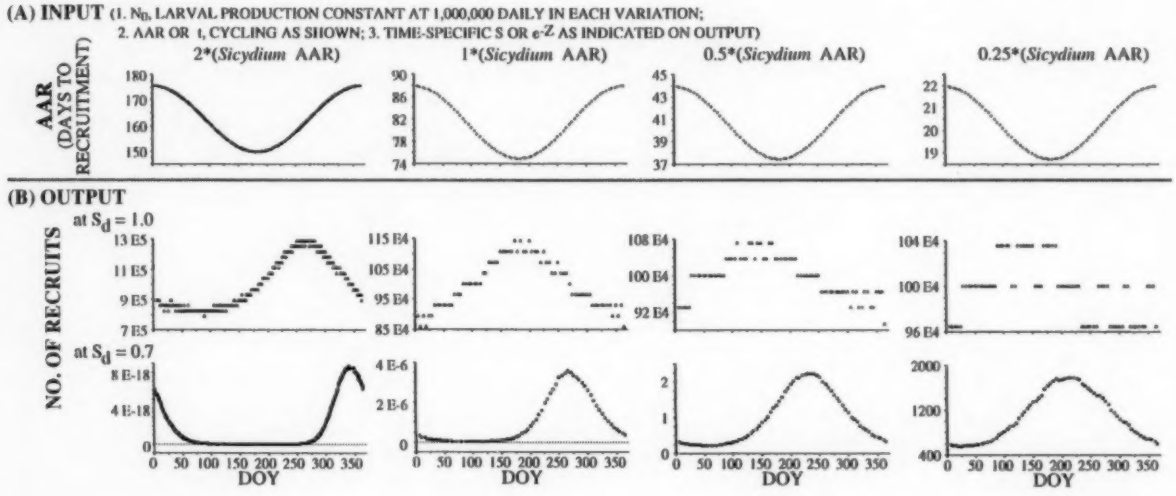
Fig. 8. Effect of scaling input AAR functions. Inputs and outputs represent all components of the equation $N_t = N_0 e^{-Z(t-0)}$. (A) Inputs to simulations are scaled naturally derived AAR functions, constant stage$_0$ production at 10^6·day^{-1}, and time-specific survival as indicated on each row of output graphs. (B) Output of simulations (points are 28-day running means). From left to right: vertical groups of graphs represent simulations using AAR functions equal to 2×, 1×, 0.5×, and 0.25× the fitted values for *S. punctatum* in Dominica. From top down: input AAR in days, predicted recruitment assuming zero mortality ($S = 1.0$, $Z = 0$), and predicted recruitment assuming 30% mortality per day ($S = 0.7$, $Z = 0.36$).


(A) INPUT (1. N0, LARVAL PRODUCTION CONSTANT AT 1,000,000 DAILY IN EACH VARIATION;
2. AAR OR t, CYCLING AS SHOWN; 3. TIME-SPECIFIC S OR e-Z AS INDICATED ON OUTPUT)
AAR (DAYS TO RECRUITMENT)
2*(Sicydium AAR)
1*(Sicydium AAR)
0.5*(Sicydium AAR)
0.25*(Sicydium AAR)
(B) OUTPUT
NO. OF RECRUITS
at Sd = 1.0
at Sd = 0.7
DOY

prediction method just described generally finds the center of the trough. A log y axis helps to show this. Relative variation (range/minimum) increases at higher mortality. The lower values occupy large ranges on logarithmic scales although they are (arithmetically) extremely close.

At low mortality rates and where the AAR function is sinusoidal, the maxima and minima of recruitment will be approximately one-half cycle apart because they depend on the derivatives (or nodes). At higher mortality this is less true, especially in sinusoidal curves with high dAAR/dt, because as amplitude of the curve increases, the maximum and minimum AAR approach the same diagonal (recruitment trajectory with slope of –1) so that the minimum recruitment can be very close to the maximum.

Effectiveness of the modified Doppler equation

The comparison of results (Fig. 10) of numerical simulation with those of the modified Doppler equation (eq. 3) are for an HDR model (i.e., assuming that AAR is determined at hatch). The results are virtually identical, supporting the analogy of this recruitment-dynamic phenomenon with the Doppler effect. The modified Doppler equation is more convenient than numerical simulation and can be set up in a spreadsheet to generate predictions, provided |dAAR/dt| stays smaller than 0.5. This caution is because, when |dAAR/dt| exceeds about 0.5, recruitment is underestimated, and furthermore when dAAR/dt > 1.0 (in the Doppler analogy this is where approaching velocity V exceeds C), a negative (nonsensical) number is calculated for F_p. When in doubt, results should be checked by numerical simulation.

Note that simulation explicitly deals with individuals, which then require summing over each recruitment time unit of interest (e.g., each day). In contrast, the modified Doppler equation treats hatch and recruitment as frequencies or rates, so that summing is inappropriate.

Conclusion and implications

Where AAR changes, simple arithmetic ordains that it will influence recruitment (N_t); the eventually realised recruitment variation is a summation of the effects (eqs. 1–3, 5) of N_0, Z, AAR, and also (new in this paper) the rate of change of AAR over time (dAAR/dt). Therefore, attempts to explain recruitment variations should consider the existence of temporal change in AAR. For spawning (N_0) variation to dominate in determining recruitment, it must exceed the variation in recruitment caused by AAR variation at the prevailing level of mortality, likewise for temporal variation in mortality.

The link of changes in AAR with changes in recruitment suggests how subtle changes in either abiotic or biotic environmental factors, which affect growth rate and AAR, can be amplified into recruitment surplus or failure. Thus they may help account for seasonally skewed recruitment of tropical species and the modal timing of life-history events within seasons in nontropical species.

The theory gives indirect access to parameters difficult to measure in the field: using the model (via simulation or via the modified Doppler equation) to construct predicted recruitment, observed recruitment can in principle be subtracted to give residuals. Residuals could be used to detect and, to some extent, describe temporal patterns not included in inputs to the model. For example, by elimination, if the temporal structure is adequately described in two of the three input variables (N_0, Z, or AAR), then the bulk of the unexplained (residual) temporal pattern would give insight to pattern in the less-known variable. The dynamics generated by temporal variation in AAR do not depend on sinusoidal or any particular pattern of temporal structure; any structure that can be described either

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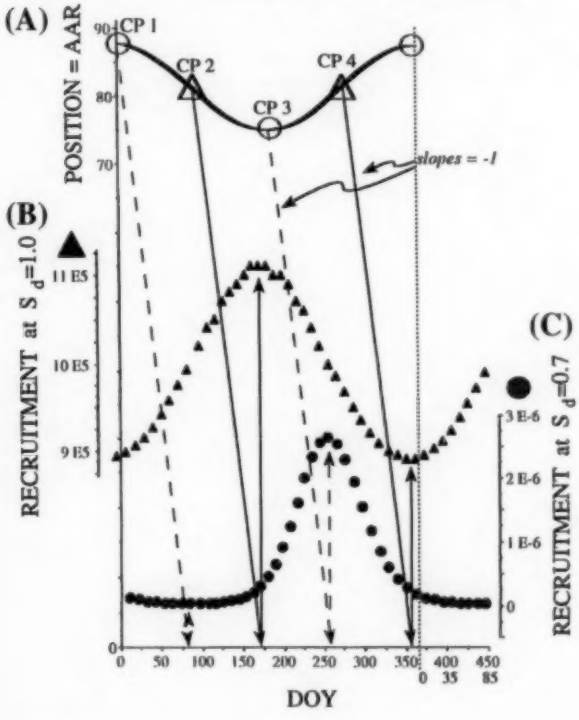
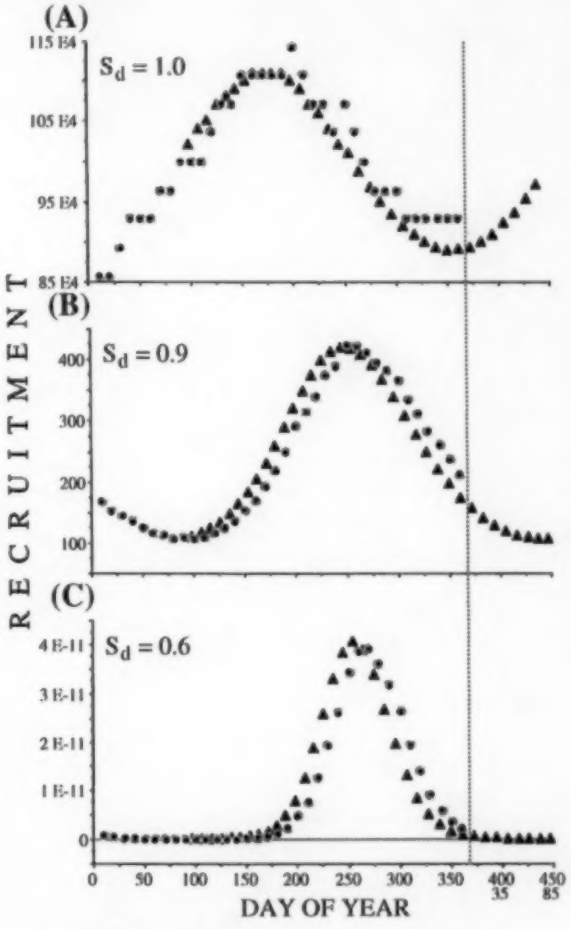
Fig. 9. Graphic estimation of recruitment peak timing from the AAR curve. Examples are for time-specific mortality low (B, ▲) or high (C, ●). Part A: AAR as in Fig. 7. Cardinal points CP1 and CP3 are maximum and minimum AAR (open circles), and CP2 and CP4 are the most negative and most positive dAAR/d*t* (open triangles). (B and C) Recruitment (as individuals per unit time) profiles from the HDR model using modified Doppler equation at $S = 1.0$ and $S = 0.7$. The long arrows angled downward from cardinal points are larval trajectories (lines with slope = –1), and their x values at $y = 0$ give timing (x value) of recruitment peaks and troughs. The derivatives (CP2 and CP4) are useful in predicting recruitment peaks at very low mortality, while the simple extremes of AAR (CP1 and CP3) are useful in predicting the peaks at high mortality. At intermediate survival rates the peak positions also will be intermediate but cannot readily be determined by geometry.



as a function, or even as a list, of AARs over time can be used in equations or simulation.

The logic presented in this paper is not limited to fish and applies to any definition of recruitment or to the duration of any stage, whether it coincides with a biological milestone (e.g., settlement, metamorphosis, germination, flowering, emergence, etc.) or not (e.g., size). The main limitation with many organisms may be the difficulty of determining AAR. Young fish record daily growth as incremental growth of otoliths, and many bivalves set down growth lines that can be used to determine age (e.g., Evans 1972), but in species without such growth-recording structures, individuals can sometimes be individually marked and followed to estimate the variability of time required to complete important life-history milestones.

Fig. 10. Comparing predictions using the modified Doppler equation (eq. 3; ▲) and the numerical simulation program (●). Panels A, B, and C compare modified Doppler and simulation results for $S = 1.0$, 0.9, and 0.6, respectively. Results are virtually identical. (However, although the match is excellent at the moderate AAR slopes here, caution is advised if curves contain high |dAAR/d*t*| (about 0.5 or more) because the accuracy of the modified Doppler equation may diminish.) Numerical simulation results were smoothed using a 28-point running mean, which introduces a lag of ~14 days relative to the results of the modified Doppler equation.



● Calculated by numerical simulation
▲ Calculated by Doppler equation

In fishes with seasonally restricted spawning periods, changes in AAR would still affect the similarity of the recruitment pattern to the production pattern. Failure to acknowledge AAR variation could prevent identification of the most important spawning periods. Lags from hatch to recruitment (AAR) should not be assumed constant (e.g., Robertson et al. 1988; Robertson 1990), because if this assumption is false, inference of peak spawning times from recruitment pattern will be unreliable.

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On evolutionary time scales, an AAR curve that is stable (cycle-to-cycle) may select for preferential allocation of reproductive effort to certain seasons to take advantage of the AAR seasonal minimum. This is not equally physically possible among species: preferential allocation would be less expected in smaller fish owing to their higher annual total egg mass/body mass ratio (Burt et al. 1988). Long-term variations in the AAR cycle's mesor or its amplitude could reflect long-term climatic or environmental variation. Spawning at times or seasons other than at the AAR minimum may be selected for if competition at an over-subscribed AAR minimum diminishes its advantages, but a shorter AAR would still be an attractor according to its rank (fitness benefit) among other attractors (biotic or abiotic), which may be synchronous or not. In other words, AAR variation would consitute a part of the landscape of selective force that forms reproductive peaks, but it could be masked by, or could mask, others. The hypothesis of preferential allocation of reproductive effort toward the AAR minimum could be probed by looking for peaks in reproduction at times inconsistent with other variations that might influence reproduction directly (such as food availability and temperature) in the adult habitat, yet consistent with optimization of AAR in progeny.

Although it is mathematically unavoidable that AAR plays a large role in recruitment, generating 10- to a 100-fold variation at plausible time-specific survival rates, the importance of that role relative to other factors, which also may have large effects, remains a question. For *S. punctatum* (Fig. 1E), the resemblance of observed phase relationships (temporal locations of minima and maxima in AAR and yield) to model output (Fig. 7) supports seasonal shifts in AAR as being capable contributing greatly to observed seasonal recruitment variation. If other factors also contribute to the temporal structure of fishery variation they must either be small or nearly balance each other, because the predicted curves are already quite similar to the observed fishery. The relative contribution of other factors remains to be determined for *S. punctatum*.

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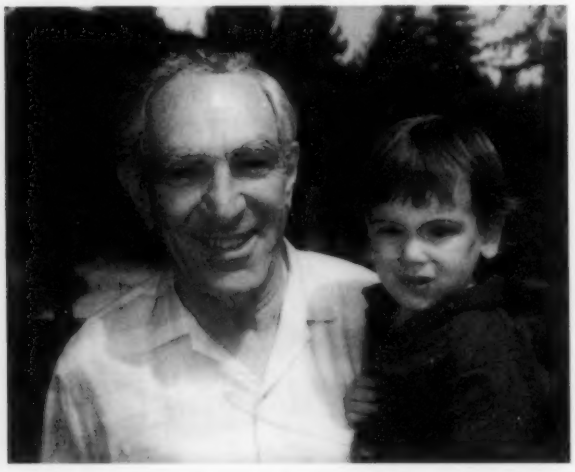
OBITUARY / NÉCROLOGIE

Leo Margolis, O.C., Ph.D., F.R.S.C. 1927–1997



The worlds of parasitology, fisheries biology, aquatic health, scientific diplomacy, and amateur hockey have lost a good friend and brilliant scientist with the death of Dr. Leo Margolis on January 13th, 1997. Leo collapsed from a heart attack at the exit from the Pacific Biological Station as he was walking home at 6 p.m. on the day before his scheduled retirement. He died 5 days later in hospital, surrounded by his family.

Leo's death was far too soon and unexpected for all of his widespread friends and family, but it seems entirely characteristic of the man that he should fall midway between the two poles of his life: his family and the Biological Station where he had carried on his professional duties over a span of 46 years.

The facts are easily listed. Leo Margolis received a B.Sc. (Cum Laude) in zoology and chemistry in 1948, a M.Sc. (Magna Cum Laude) in bacteriology and immunology in 1950, and a Ph.D. (Magna Cum Laude) in parasitology in 1952, all from McGill University. Leo and his wife Ruth then moved west to Nanaimo, where he began an exemplary career as government scientist, advisor, diplomatic representative, and mentor to many scientists at the Pacific Biological Station and around the world. Along the way, he raised a remarkable family, of which he was quietly but justifiably proud.

Leo's ensuing scientific interests and contributions to Canada, and to British Columbia in particular, have been of tremendous depth. As well as his work on the sea life of Pacific salmonids, he studied the parasites and diseases of cultured and wild freshwater and marine fishes and their control; ecology of parasites of aquatic animals; parasite transfaunation and introduction through river diversions; and questions concerning public health and the economic importance of fish parasites. In total, Leo's research led to some 150 primary scientific papers and many more technical reports, forming an indispensable body of knowledge for everyone involved in problems caused by parasites of aquatic animals.

Although his worldwide scientific fame is founded on his achievements in parasitology and fish health, some of his most important contributions come from his work in international trade and fisheries negotiations. It is likely that few of Leo's many friends realized the breadth of his career.

Leo first attended the meetings of the International North Pacific Fisheries Commission in 1956 and played important roles in almost all meetings since then. One of Leo's first major scientific contributions occurred in the 1950s, when he developed a method of distinguishing between North American and Asian salmon stocks using parasites. At the time, the need for a method to prove that Asian high seas salmon fisheries were intercepting North American salmon was of pressing international importance.

Leo's now-classical research used naturally occurring freshwater parasites to demonstrate the continent of origin of salmon caught in mixed-stock high seas fisheries. This work formed much of the basis for international negotiations that gradually moved Asian salmon fisheries westward over the years, minimizing or eliminating interceptions by foreign salmon fisheries on North American stocks.

After extended negotiations, the International North Pacific Fisheries Commission (INPFC) was replaced in 1992 by the North Pacific Anadromous Fish Commission (NPAFC), a revised organization which now includes Russia. The change in Commissions was partly needed because years of scientific work at INPFC had successfully resulted in the elimination of all directed fisheries for salmon on the high seas apart from scientific research programs. The "Fisheries" that formed part of INPFC's name and original focus no longer existed. The new organization is focused much more closely on scientific questions concerning the ocean biology of salmon, a subject that Leo viewed as critical to improving our understanding of the productivity of salmon stocks. Leo was elected first Chairman of the Standing Committee on Scientific Research and Statistics, a position reporting directly to the president of NPAFC. Thus both the name and the rationalization of north Pacific salmon fisheries owe much to Leo.

It was also largely through Leo's initiative and energy that Canada developed its Fish Health Protection Regulations, without which British Columbian fish culture and fish farming industries would probably not be nearly as successful as they currently are. As a result, Leo also made pivotal contributions to the elimination of non-tariff barriers on the importation of Canadian salmon to foreign markets. Canadian salmon are barred from import to some countries except as canned product, allegedly because of concerns over inadvertent introduction of non-native fish diseases. In Canada's view, these

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regulations are a non-tariff barrier to trade and are without scientific foundation.

Trade negotiations between countries require meticulous preparation and careful coordination among many groups. Over 20 years were needed to negotiate the elimination of these tariffs, with Leo remaining the central technical expert because of his knowledge of fish diseases and his international reputation. Finally, in 1995, Leo and colleagues convinced the New Zealand animal health authorities that there was no biological basis for excluding any form of Canadian salmon from import to New Zealand. It will be a fitting tribute if Australia also reaches the same conclusion, prior to Canada taking the case to GATT.

Leo's meticulous approach was equally reflected in his exemplary performance of administrative duties, duties that many scientists roll their eyes at, but which are critical to the functioning of the system we work within. He became Head of what is now called the Fish Health and Parasitology Section of the Pacific Biological Station in 1967, acted as Assistant Director in 1969–1970 and 1972, and was acting Director in 1977 and 1986–1987. He was appointed Senior Scientist of the Biological Station in 1990.

Leo was elected in 1975 to the Fellowship of the Royal Society of Canada and was awarded the R.A. Wardle Medal in 1982 by the Parasitology Section of the Canadian Society of Zoologists in recognition of his "outstanding contributions to the advancement of our understanding of the biology of parasitic organisms." He was an active member of six scientific societies and served as Chairman of the Parasitology Section of the Canadian Society of Zoologists and then as Vice President (1989–1990) and President (1990–1991) of the Canadian Society of Zoologists. He served on the editorial boards of five journals and had just completed co-editing the second major volume of a multi-volume set planned on the biology of the Pacific salmon.

Most recently, he was made an Honorary member of the British Society for Parasitology in 1994, awarded the American Society of Parasitologist's Distinguished Service Award in 1995, and awarded an Honorary D.Sc. from St. Mary's University, Halifax, in 1996. A commemorative "Festschrift" was published in his honour by the Canadian Journal of Fisheries and Aquatic Sciences in 1995. As Bob Kabata, Leo's longtime colleague, remarked at his memorial service, scientists from six countries have to date named 11 species of fish parasites after him, an honour that speaks volumes about the man as much as the scientist.

Leo's many activities reflect the influence that scientists can make to society at large, as well as within their own sphere of expertise. Leo was invested as an Officer of the Order of Canada in 1990 in recognition of not only of his scientific achievements but also his contributions to Canadian society as a private citizen. A keen supporter of amateur hockey, first as a player and then through the British Columbia Amateur Hockey Association, he was its President from 1963 to 1966, the youngest Canadian ever to hold such a position in any province. In recognition of his past services, the Association made him an Honorary Vice-President (1968–1988) and then an Honorary Life Member (1988). In 1989, he received the B.C. Amateur Hockey Association's highest honour, the Diamond Stick Award, for outstanding services to amateur hockey in B.C. and, in 1990, the Canadian Amateur Hockey Association granted him its Western Order of Merit Award.

In nominating Leo for consideration as a Life Member of the Canadian Hockey Association, the British Columbia Association summed up his contributions as follows: "*No individual has contributed so much, so unselfishly over such a long period of time for the advancement of hockey in our Branch and across Canada.*" Leo's mark on amateur hockey in B.C. alone would make for a remarkable contribution to society quite apart from his many scientific contributions. Indeed, the description is equally apt in describing Leo's contributions to science.

Although the facts describing Leo's life are easily listed, the richness of the life leading to his contributions is much more difficult to convey. To all of us at the Biological Station, Leo was an outstanding individual and model scientist and public servant. In the course of 46 years of continuous service to the government and the people of Canada, Leo reached a position of high distinction and respect both nationally and internationally, serving effectively as both a scientist and a diplomat, and as a leader for young British Columbians involved in amateur hockey. Yet for all of Leo's remarkable achievements, he did this while remaining a thoughtful and remarkably self-effacing individual, whose integrity and carefully couched advice was valued around the world.

Leo's industriousness and dedication provide a model worthy of emulation by all of us, and I can think of no better example of the quiet but essential role that science and scientists can play within government and society. Leo's life reflects an individual who distinguished himself not only in his achievements, but also by earning the respect and admiration of his superiors, colleagues, and friends while doing so. When combined with the remarkable family that Leo raised and left as his most lasting legacy, I can think of no better role model of the ideal "diplomat–scientist" and private citizen. Leo is survived by his wife Ruth, four children Rhonda, Robert, Murray, and Conrad, and four grandchildren, Zachary (in the photograph), Leah, Taro, and Hannah, plus a network of friends that stretches quite literally around the world. Leo and his legacy will be remembered for many years to come.

David W. Welch
Pacific Biological Station
Nanaimo, B.C.

The Leo Margolis Scholarship

This scholarship has been established as a memorial to Dr. Leo Margolis, an internationally preeminent parasitologist and a staunch supporter of the Canadian Society of Zoologists since its inception in 1961. The competition for the scholarship is open to any Canadian registered in a graduate studies program at a Canadian university at the time the scholarship is taken up and whose research is in the field of fisheries biology.

The stipend of the scholarship may vary from year to year but will represent the interest earned by the Leo Margolis Endowment Fund, registered in the Zoological Education Trust within the Canadian Society of Zoologists. The scholarship may be held for up to 2 years and preference will be given to applicants whose research will be oriented towards the Pacific coast fauna.

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The Canadian Society of Zoologists will select the successful applicant using the chairperson (or suitable representative) of each of the three Sections within the Society as the selection committee. The awardee will be announced by the Canadian Society of Zoologists at the annual general meeting for that year.

Contributions to the Fund should be sent to the Treasurer of the Canadian Society of Zoologists (Attention: Dr. J. Fenwick), Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5 and made payable to the Canadian Society of Zoologists (Leo Margolis Endowment Fund). Donations are considered charitable for Canadian income tax purposes.

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ANNOUNCEMENTS / ANNONCES

CANADIAN CONFERENCE FOR FISHERIES RESEARCH (CCFFR)

Queen's University, Kingston, Ontario, 2–4 January 1998

The **major themes** of the 1998 Conference will be:

1) Interaction between terrestrial habitat and aquatic/fisheries habitat (e.g. fisheries/forestry);
2) Experimental management and decision processes for environmental/fisheries problems;
3) Indicators of fish health for use in environmental monitoring;
4) **Joint CCFFR/SCL session** — Long-term studies in freshwater systems.

Papers addressing the major themes are encouraged, though submissions on other themes related to fisheries and aquatic habitat are also welcome. CCFFR will run only two concurrent sessions in 1998. This, combined with the probable large number of submissions, will mean that the Program Committee will have to limit the number of paper presentations. Space for posters is extensive and time will be allocated for poster discussion. Please consider whether your paper could be a poster and so indicate in your abstract submission.

Unlike recent Conferences, CCFFR 1998 will be held from Friday evening to Sunday, instead of Thursday to Saturday. To ensure best use of time at Queen's University, CCFFR lunch will be provided at the University Conference Facility, and payment for lunch will be obligatory for those registering for the conference. Registration fees will be as in 1997, and lunch will be in the range of $9 – $11 all inclusive for each of the two days.

The **deadline for abstracts** is 12 September 1997.

Please follow the guidelines for hard copy, and submit abstracts to:

Dr. Roger H. Green
Department of Zoology, University of Western Ontario
London, ON N6A 5B7
Telephone and Fax: 519-661-3127

Also, send an ASCII (text) version of your abstract by e-mail to:

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Hard copy of Abstract:

1) Abstracts will be reproduced as submitted; please follow the suggested format.
2) Type within a 10 × 15 cm space, using single space typing (12 pitch).
3) Place an asterisk (*) after the name of the person presenting the paper.
4) Indicate whether the presentation will be oral (O; only standard 5 × 5 cm slide projector and overhead projector will be provided free of charge) or poster (P) after the title.
5) Please indicate, on a separate line after the end of your abstract, if your presentation COULD be a poster even if your preference is for oral presentation. Also indicate on this line to which theme (1–4) your presentation is most closely related.
6) Suggested **format:**

   RELATIONSHIPS BETWEEN SUNSPOTS AND SEA STARS (O)

   Gretzky*, W., and J. Villeneuve. Department of Epidemiology, Eastern University, Yoho, Saskatchewan.

   Sea stars shine increasingly brightly as the sunspot cycle progresses.....

   Could be poster; theme 3.

CONFERENCE CANADIENNE DE LA RECHERCHE SUR LES PECHES (CCRP)

Université Queen's, Kingston (Ontario), les 2–4 janvier 1998

Les **principaux thèmes** abordés lors de la Conférence de 1998 seront :

1) Les interactions entre les habitats terrestres et les habitats aquatiques ou du poisson (ex : pêches/foresterie);
2) Gestion expérimentale et processus décisionels pour les problèmes des pêches ou de l'environnement;
3) Les indices de la santé des poissons pour utilization dans le suivi environnemental;
4) **Session Conjoint CCRP/SCL** — Les études à long terme dans les systèmes d'eau douce.

Des contributions reliées aux thèmes principaux sont particulièrement sollicitées, mais des contributions sur d'autres thèmes reliés aux sciences halieutiques et à la biologie des habitats aquatiques sont également bienvenues. La CCRP n'aura que deux sessions concurrentes en 1998, non pas trois. Vu le nombre important de soumissions anticipées, le Comité du programme devra limiter le nombre de présentations orales. Il y aura beaucoup d'espace pour les affiches et de temps alloué pour discussion des affiches. Considérez si votre soumission pourait être une affiche et si oui notez ceci en soumettant votre résumé.

Exceptionellement en 1998 la CCRP se tiendra du vendredi soir au dimanche, non pas de jeudi au samedi. Afin d'assurer un emploi de temps efficace le déjeûner sera fourni à la Conférence et le paiement pour le déjeûner sera obligatoire pour les participants enregistrés. Les frais d'enregistrement seront les mêmes qu'en 1997 at le prix du déjeûner sera environ $9 – $11 tout compris pour chacun des deux jours.

La **date limite pour soumission des résumés** est le 12 septembre 1997.

Veuillez respecter les lignes directrices pour la copie sur papier, et soumettre au :

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5) Veuillez indiquer sur une ligne à part après la fin de votre résumé si votre présentation POURRAIT être par affiche même si vous préférez une présentation orale. Indiquer également sur cette ligne à quel thème votre contribution est le plus reliée (1–4).
6) **Format** proposé :

   PREDATION PAR LES EUPHAUSIACES SUR LES BEBES PHOQUES (O)

   Villeneuve*, J., et W. Gretzky. Departement de planctonologie, Université de Trois Pistoles, Trois Pistoles (Québec).

   Une augmentation subite de la mortalité des bébés phoques s'est avérée....

   Pourait être affiche; thème 3.

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